









# PHYSIOLOGICAL REVIEWS

VOLUME 25

1945



# PHYSIOLOGICAL REVIEWS

VOLUME 25

BALTIMORE, MD  
1945

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JOHNSON REPRINT CORPORATION  
New York, New York



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# PHYSIOLOGICAL REVIEWS

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No 1

## EFFECTS OF OXYGEN AT INCREASED PRESSURE

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Shortly after having isolated  $O_2$  Priestley (1775) wrote, "From the greater strength and vivacity of the flame of a candle, in this pure air, it may be conjectured, that it might be peculiarly salutary to the lungs in certain morbid cases But, perhaps, we may also infer from these experiments, that though pure dephlogisticated air might be very useful as a *medicine*, it might not be so proper for us in the usual healthy state of the body for, as a candle burns out much faster in dephlogisticated than in common air, so we might, as may be said, *live out too fast*, and the animal powers be too soon exhausted in this pure kind of air A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve The feeling of it to my lungs was not sensibly different from that of common air, but I fancied that my breast felt peculiarly light and easy for some time afterwards Who can tell but that, in time, this pure air, may become a fashionable article in luxury Hitherto only two mice and myself have had the privilege of breathing it "

The study of the influence of  $O_2$  on the animal organism and the view that while  $O_2$  could be used to advantage therapeutically such use might be attended with some danger, had its inception, then, in the experiments of Priestley But it is Lavoisier who is not infrequently credited with having been the first to recognize the possible noxious effects of breathing  $O_2$  The reason, for this, may perhaps be found in an erroneous interpretation which he made as a result of his having fallen into the company of physiologists At least Foster (1901) suggests it was the association with Seguin which was responsible for Lavoisier's conclusion that the site of oxidations in the body was the lungs This misinterpretation persisted tenaciously in spite of the evidence and argument to the contrary presented by a number of investigators, even for some time after the epoch-making blood gas studies of Magnus in 1837 which showed that the site of oxidations must be in the tissues as Mayow had maintained more than a century and a half before

It is not surprising, therefore, that the belief should have arisen that increasing concentration of this incendiary substance in the lung caused severe pulmonary damage Later experimental findings served to temper the more extreme of these interpretations and after a few cyclic swings the pendulum of opinion seemed to have reached a position of stability pointing to a goodly amount of evidence that breathing pure  $O_2$  or hyperoxygenated air will cause not only general physiological

changes, but also pathological alterations. In very recent years numerous reports based largely on extensive use of  $O_2$  on human subjects have appeared which maintain that the noxious effects induced in experimental animals by  $O_2$  has been overemphasized and that breathing  $O_2$  in high concentrations at atmospheric pressure is relatively harmless. The available evidence does appear to indicate that men may be somewhat superior to mice—at least in their ability to withstand the deleterious effects of breathing  $O_2$ —but this does not dismiss  $O_2$  as innocuous.

Naturally enough by far the greater part of the literature and investigative work concerning the effects of  $O_2$  has been confined to increased  $O_2$  tensions at atmospheric pressure. But the increasingly high pressure conditions to which man has found, and in the future is likely to find it expedient to expose himself either for industrial or therapeutic purposes, has called for a consideration of the effects of  $O_2$  at tensions distinctly in excess of that equivalent to one atmosphere.

Increased  $O_2$  tension in a respiratory gas may be attained by several methods (1) by the use of either hyperoxygenated air or pure  $O_2$  at normal barometric pressure, thus providing  $O_2$  tensions up to that equivalent to 100 per cent of one atmosphere, (2) by the use of normal air under compression, in which case  $O_2$  tensions in excess of one atmosphere may be attained, (3) by the use of hyperoxygenated air or pure  $O_2$  under increased pressure which provides another means of attaining very high  $O_2$  tensions. There is no sharp line of demarcation between the effects of  $O_2$  tensions just below 760 mm Hg and those above 760 mm Hg but tensions of  $O_2$  much in excess of this do introduce factors not operative to an appreciable extent where the  $O_2$  tension is less than 760 mm Hg. In addition to this, the fact that a desired increase in  $O_2$  has been achieved in so many experimental investigations by the compression of normal or hyperoxygenated air, calls for a consideration of some of the possible complicating influences which such methods introduce. For these reasons, and as a matter of convenience, the material covered below is presented under the following main headings: I Effects of Increased Oxygen Tensions Not in Excess of One Atmosphere, as Induced by Pure Oxygen, Hyperoxygenated Air or Normal Air at Increased Pressures, II Possible Complicating Variables Introduced by Compressed Air or Artificial Gas Mixtures, III Effects of Oxygen Tensions in Excess of One Atmosphere as Induced by Pure Oxygen, Hyperoxygenated Air, or Normal Air at High Pressure. The oxygen tensions considered in Part III will be referred to as oxygen at high pressure (OHP).

I EFFECTS OF INCREASED OXYGEN TENSIONS NOT IN EXCESS OF ONE ATMOSPHERE, AS INDUCED BY PURE OXYGEN, HYPEROXYGENATED AIR, OR NORMAL AIR AT INCREASED PRESSURES. *Metabolism.* Seguin and Lavouier (1789) performed experiments from which they concluded that breathing pure  $O_2$  did not alter any of the vital processes, the respiration and circulation were neither accelerated nor retarded and the temperature remained unchanged. These results were supported by the experiments of Regnault and Reiset (1849) in which animals (rabbit and dog) breathing  $O_2$  in concentrations of from 46.63 per cent to 72.38 per cent for as long as 23 hours showed no change either in the  $O_2$  consumption or in the respiratory quotient from that obtaining under normal conditions.

Birch (1859) reported, however, that the temperature of healthy animals was usually first increased then decreased while breathing  $O_2$ . Clinical observation, he thought, afforded evidence that  $O_2$  when employed in disease might raise or lower the temperature under different circumstances but that only in rare instances did it exert any influence on persons enjoying perfect health. Smith (1870) disagreed with the finding of Regnault and Riesel and reported that in experiments upon himself there was an initial decrease in  $CO_2$  exhaled, yet he was of the opinion that ultimately there was a slight increase in the  $CO_2$  output. His short administrations of  $O_2$  raise the question of the validity of his results.

The studies of Speck (1879) made on men, led him to support the conclusions of Regnault and Riesel. Lukjanow's data (1884) obtained from rat, cat, dog, guinea pig, dove and canary showed considerable variation, in many instances the  $O_2$  uptake in  $O_2$  rich atmospheres was increased but more than half of the experiments showed either no change or a decrease. His data are not convincing but they were interpreted as confirming the view of Regnault and Riesel. Saint-Martin (1884) on equally questionable data arrived at a similar conclusion that the chemical phenomena of respiration were not appreciably changed by breathing hyperoxygenated air. Wood and Cerna (1890) also claimed that the breathing of  $O_2$  was without effect on the organism but in view of their limited number of experiments and the manner of  $O_2$  administration such claim would appear to be hardly justified, especially where small changes may be involved.

Bert (1878) using methods similar to those of Regnault and Riesel found that when the animals were breathing  $O_2$  mixtures equivalent to 48.7 per cent, their  $O_2$  consumption was higher than when breathing mixtures of either higher or lower  $O_2$  content (87.5 per cent and 20.96 per cent respectively). This led him to the conclusion that there was an optimum  $O_2$  percentage for oxidations. "*L'activité des combustions organiques a donc été en augmentant d'abord, pour diminuer ensuite après avoir passé un certain maximum qui est probablement placé au-dessus de 2 atmosphères*"

Quinquaud (1884), interested in the therapeutic use of  $O_2$ , carried out a number of experiments chiefly on dogs, in most of these the  $O_2$  was inspired from a balloon and expired into the free air (or into another balloon if analyses were desired). From these studies, and from a comparison of the gas content of arterial and venous bloods he drew the following conclusions: that it is possible to superoxygenate the blood by breathing pure  $O_2$  so that an additional 2.2 volumes per cent is carried in arterial blood, that as a result of such  $O_2$  breathing, there is a decrease in rectal temperature, a slight decrease in  $CO_2$  exhaled, a diminution of organic combustion, and that  $O_2$  has a sedative effect. These changes, he said, were analogous to, but less accentuated than those observed by Bert.

Fredricq (1884) reported that in experiments performed on himself and on rabbits there was no increase in the  $O_2$  consumption when  $O_2$  rich mixtures were breathed, but with  $O_2$  poor mixtures, it was diminished. No data are given in his report, but he emphasizes that when the subject is changed from breathing ordinary air to  $O_2$  enriched air, or to pure  $O_2$ , there occurs during the first few minutes an augmentation in the absorption of  $O_2$  due to its solution in the blood and lymph, but after equilibrium is attained between plasma and pulmonary air, the

absorption of  $O_2$  returns to its normal value, a conclusion which had been previously arrived at by Speck.

Some few years later Loewy (1894) concluded from his experiments that the respiratory gas exchange is independent (within wide limits) of the  $O_2$  content of the respired air. Doubling the normal  $O_2$  content of inspired air or decreasing it even to a point where an alveolar tension of 40 mm Hg was attained, did not change the  $O_2$  consumption or the  $CO_2$  exhaled. In summarizing the results of his experiments in which the  $O_2$  content of the inspired air had been increased to 37.7 per cent and 49.28 per cent, he reported that the metabolism was not altered, and the  $O_2$  consumption showed no notable difference from normal. He disagreed with Bert's observations concerning an optimum  $O_2$  percentage for oxidations.

Terray (1897) likewise reported that breathing of  $O_2$  rich atmospheres did not appreciably alter the total metabolism of experimental animals and that with  $O_2$  percentages of from 10.5 to 87, the metabolism was independent of the  $O_2$  content of the air breathed, but along with his conclusion that metabolism was unchanged, Terray reported that the  $CO_2$  output was diminished to some degree.

The consensus of opinion at about that time (Tigerstedt, 1902) was that breathing  $O_2$  or hyperoxygenated air did not alter metabolism but an examination of the data presented by some investigators in support of that opinion reveals that the experimental evidence was not as convincing as was implied.

Rosenthal (1898, 1902) reported that breathing of  $O_2$  rich mixtures caused a distinct and quite large increase in the uptake of  $O_2$  and that there was a storage of  $O_2$  in the tissues. These surprising results and conclusions were refuted by Durig (1903) and Schaternikoff (1904) who, apparently stimulated by Rosenthal's claims, had been working independently and with different methods in Vienna and Moscow respectively. Durig's experiments on both animals and men gave him data which led him to conclude that the  $O_2$  consumption was not increased by breathing hyperoxygenated air and he found no evidence of  $O_2$  storage in the tissues. Schaternikoff, in an attempt to determine if Rosenthal's claims, which had been arrived at by the use of small experimental animals, held also for man, was likewise unable to find any indication that the  $O_2$  consumption was dependent upon the  $O_2$  content of the inspired air.

Hill and Macleod (1902a), referring particularly to the reports of Panum (1868) and von Liebig (1878) that increase of atmospheric pressure had no distinct influence on the  $O_2$  uptake or  $CO_2$  output in man, and the report of L. Smith (1897a) that the  $O_2$  tension in the blood was lowered by prolonged breathing in an atmosphere of pure  $O_2$ , remarked that the data up to that time were such that "Nothing conclusive can be asserted as to the influence of  $O_2$  on the gaseous metabolism." Their own experiments, however (Hill and Macleod, 1902a, 1903c), showed that breathing pure  $O_2$  at atmospheric pressure caused a decrease in the  $CO_2$  exhaled and in the  $O_2$  absorbed and a drop in body temperature. These changes did not occur immediately but were usually quite distinct in 30 minutes. It was concluded that breathing  $O_2$  does cause a distinct diminution in metabolism.

The same investigators (Hill and Macleod, 1903a, b, c) found that compressed

air at 4 atmospheres and upwards also diminished the  $\text{CO}_2$  output and lowered the body temperature and that these effects were most pronounced at the higher pressures. The British Admiralty report (Hill, 1912) indicated that the  $\text{CO}_2$  output in divers during rest was not altered by breathing compressed air, the resting output in one subject was the same at a depth of 210 feet as it was at 6 feet. Hill and Macleod (1903c) state, "We observed like Bert that prolonged exposure to even one atmosphere of pure oxygen slightly lowered the  $\text{CO}_2$  output and temperature of mice, both returning to normal on replacing the oxygen with air. On increasing the tension of  $\text{O}_2$  we find a general increase in toxic effect, but no constant relationship owing to differences in individual susceptibility of mice." But these authors could not confirm Bert's conclusion that compressed air diminished the  $\text{CO}_2$  output in proportion to the contained partial pressure of  $\text{O}_2$ , and they suggested there were factors, other than the partial pressure of  $\text{O}_2$ , to be considered, such as the cooling effect of compressed air due to its increased conductivity and saturation with water vapour, the increased resistance offered to movement of air through the respiratory passages and the altered diffusion of  $\text{CO}_2$  from the alveolar air. However, the finding that the body temperature was lowered, was taken as clear proof by these investigators that the lowered  $\text{CO}_2$  output was due to a decrease in oxidation rather than to any interference with release of  $\text{CO}_2$  into the alveoli which might be caused by possible alteration in lung epithelium.

Hough (1910), in experimental studies carried out on human subjects breathing  $\text{O}_2$  in 60 to 80 per cent mixtures, questioned the "soundness of the usual view that the consumption of oxygen by the cell is determined entirely by the cell and is independent of the quantity of oxygen provided by the blood." Benedict and Higgins (1911) felt that while the reports up to that time favored the view that increased  $\text{O}_2$  in the respired air does not significantly alter metabolism, they were not entirely convincing and maintained that the question was "not yet definitely settled." In an attempt to get more detailed and reliable data these authors carried out a very careful experimental study on healthy men. Their determinations were made with the experimental subjects lying at complete muscular rest 12 hours after the last meal. The  $\text{O}_2$  concentrations employed were 40, 60 and 90 per cent mixtures in air, but the duration of administration was relatively short, usually not more than 15 minutes, so that the full effects of the increased  $\text{O}_2$  may not have had time to develop in every instance. The results of these tests led to the conclusion that when breathing the  $\text{O}_2$  enriched air, the metabolism, as indicated by the gaseous exchange and respiratory quotient, was the same as that obtaining in ordinary air. However, in three of six subjects the  $\text{O}_2$  consumption was found to be greater in the 40 per cent mixture than in either the 60 to 90 per cent) or the lower (20 per cent)  $\text{O}_2$  mixtures, in one of these, consumption in the 40 per cent mixtures was 3 per cent above that of the 60 to 90 per cent mixture. The authors considered these increases in  $\text{O}_2$  consumption insignificant, and while they pointed out that such effect was in agreement with the report of Bert that the optimum of  $\text{O}_2$  consumption was in the neighborhood of 45 per cent mixture, they did not believe their results substantiated Bert's views.

Hill (1912c) maintained Bert's experiments were quite insufficient to justify his claim that  $O_2$  consumption increased with an increase in  $O_2$  content of the air breathed up to an optimum of about 45 per cent. Krogh (1916) likewise says Bert's results are invalid because his methods were not nearly accurate enough to demonstrate this. While admitting the determinations of Benedict and Higgins (1911) as probably the most accurate, Krogh does not fully agree with their conclusions, and maintains that if due consideration be given to the washing out of the tissues by nitrogen it "seems" that "breathing of oxygen does increase the metabolism to some slight extent, and it must be borne in mind that a large effect is not in any case to be expected, because the oxygen supply to the tissues will only be increased by the extra amount of oxygen physically dissolved in the arterial blood which cannot be more than 10 per cent of the total quantity carried."

The results obtained on warm blooded animals point, when considered in their entirety, in the opinion of the writer, towards the conclusion that oxygen pressure is practically the limiting factor for the oxidations, but that it is so regulated as to be just sufficient. A diminution of the oxygen supply to the tissues, which will take place when the oxygen pressure in the inspired air falls below something like 85 mm, causes a decrease in rate of oxidation, while an increase in oxygen pressure appears to produce a slight increase."

Campbell (1927) found that the metabolism of rabbits was not altered by their prolonged exposure (about six weeks) to air in which the  $O_2$  content was 200 per cent above normal and the barometric pressure at 750 mm. The  $O_2$  consumption remained unchanged, but the  $O_2$  and  $CO_2$  tensions of the tissue, as estimated by intra abdominal and subcutaneous bubble technique increased. The body weight decreased during the  $O_2$  exposure but gradually returned to normal after the animals' return to breathing normal air. Clark, Gaddie and Stewart (1933) obtained experimental data from isolated frogs' hearts which indicated that the R.Q. of hearts kept in  $O_2$  instead of air, was greatly reduced thereby, the carbohydrate consumption slightly diminished and the total  $O_2$  consumption was raised about 15 per cent.

Behnke, Johnson, Poppen and Motley (1935) reported that the  $O_2$  consumption of healthy men is high during the first twenty minutes of exposure to 79 per cent  $O_2$  at one atmosphere, then decreases to a constant level which is maintained for periods up to four hours. An inspection of their data shows that even after this period, the  $O_2$  consumption level was still above the calculated basal rate. Richards and Barach (1934) found that the metabolic rate of two normal young men (measured by  $CO_2$  output) was unchanged by a week's residence in an atmosphere enriched to 40 per cent of  $O_2$ .

Becker Freyseng and Clamann (1939) observed a tendency to lowering of their body temperatures during the first 24 hours of exposure to 90 per cent  $O_2$  at atmospheric pressure, but during the second day the body temperature increased—perhaps secondarily to the lung injury which the exposure induced rather than as the result of some more direct action of  $O_2$  metabolism. The same authors also found changes in alveolar  $CO_2$  tension, during the first hour it increased above normal, by the second hour it had decreased very distinctly, and except for a temporary rise at about the fifth hour it was well below normal for ten

hours of exposure Anthony (1940) is of the opinion that breathing  $O_2$  causes no change in metabolic rate

Davis (1941) finding that the administration of  $O_2$  caused an increase in the amount of carbon dioxide in the blood of normal, anesthetized animals, suggested that this might have been due to a stimulation of tissue metabolism Craig and Beecher (1943) report that the metabolism of cortex, medulla, and spinal cord slices was markedly diminished by  $O_2$  tensions below 21 per cent of an atmosphere but that only the metabolism of the cortex showed any significant difference for  $O_2$  concentrations ranging between 100 and 21 per cent It was suggested that perhaps by increasing the  $O_2$  tension of the inspired air the metabolism of the brain *in vivo* might be increased so as to be significant in relation to the toxic action of high concentrations of  $O_2$

In experiments on oat seedlings, Albaum, Kaiser and Eichel (1940) found that 50 per cent  $O_2$  increased the rate of  $O_2$  consumption but decreased the rate of growth, pure  $O_2$  however diminished the  $O_2$  consumption, slowed the growth and resulted in a marked reduction in the final length of coleoptile Stephenson and Whetham (1924) reported that exposure of *B. coli* to an atmosphere of  $O_2$  increased both the  $O_2$  consumption and the  $CO_2$  output

The available data concerning the effects of  $O_2$  on nitrogen metabolism are variable and for the most part are unsatisfactory In determinations on himself, Smith (1870) found an initial decrease in urea excretion for the first four days after beginning the  $O_2$  inhalations, followed by a return toward normal The experimental method employed, the limited number of experiments performed, and the small volume of  $O_2$  (6 to 8 gals) respired for only a short period each day render the data of doubtful value It was claimed, however, that uric acid was diminished by the daily use of  $O_2$  and that the color of urine was changed from dark to light in spite of an increase in specific gravity Bert (1878) reported that compression of himself to about 2 atmospheres of air, i e, equivalent to a partial pressure of  $O_2$  of about 40 per cent of an atmosphere, resulted in a slight increase in urea output, in dogs exposed to three atmospheres of air pressure for 9 hours on successive days, he also found a small increase in urea output, but exposure of dogs to air at 8 atmospheres for 6 or 7 hours caused a sharp decrease in urea excretion

Jammet (1871) claimed that the urea excretion of caisson workers was increased by their exposure to compressed air Snell (1896) in observation on himself found that exposure to increased air pressure caused no change in the urea excretion Terray (1897) concluded from urinary studies that nitrogen metabolism was not altered in breathing  $O_2$  enriched air Hill and Macleod (1903c) found in their experiments on dogs exposed to air pressures at six to seven atmospheres, i e,  $O_2$  partial pressures equivalent to about 120 per cent of an atmosphere, that such exposures caused no marked or constant variation in urinary constituents They considered Bert's findings inaccurate Adolph (1934) found 100 per cent oxygen increased urine formation in pithed frogs In summary it may be said that the literature provides no consistent and dependable evidence that breathing hyperoxygenated air or pure  $O_2$  causes any pronounced

alteration in metabolism as manifest by the exchange of respiratory gases and the more numerous reports still favor the interpretation of Seguin and Lavoisier that the respiratory exchange is not a function of the  $O_2$  tension. Yet such a generalization does not dismiss the frequent discrepancies which, while considered in consequential by some of the authors supporting that generalization may nevertheless be of physiological significance.

Some investigators who have concluded that metabolism is not altered by the administration of hyperoxygenated air at atmospheric pressure have reported, however, that such administration does cause alterations in pulse rate (Benedict and Higgins, 1911). This change in cardiac activity in itself, clearly indicates that breathing increased percentages of  $O_2$  alters the physiological activity of tissue, and in final analysis such alterations must involve changes in fundamental metabolic processes, even though no coincident exchange in gaseous interchange may be readily detected by the techniques usually employed.

A brief consideration of the effects of administration of  $O_2$  in concentrations below that of the normal atmosphere is of interest in this connection. The generalization that a moderate reduction of the  $O_2$  content in the respired air below the normal 20 per cent does not alter metabolism, may account in part for the erroneous interpretation that there is no physiological response to low  $O_2$  administration until the  $O_2$  content of the inspired air falls to about 13 per cent. But the experiments of Bernthal (1938), of von Euler, Liljestrand and Zotterman (1939) clearly demonstrate that even a very small decrease in  $O_2$  content causes distinct alterations in respiratory and circulatory mechanisms. Such alterations constitute further evidence of changes in fundamental metabolic processes. Furthermore the demonstrated effects of  $O_2$  on enzyme preparations (see below) provide ample reason to expect that the administration of  $O_2$  in vivo might result in metabolic alterations.

*Breathing* In spite of the general impression and statements in standard textbooks (Starling, 1936, Howell, 1940) to the effect that the inhalation of  $O_2$  enriched air or pure  $O_2$  does not alter breathing, there still crop up dissenters with experimental evidence in support of their claims to the contrary. Certainly a lack of complete unanimity of opinion can be traced back to the very earliest observations on experimental animals and men breathing  $O_2$ , hyperoxygenated, or compressed air. Some of these earlier reports were no doubt colored by views of overzealous therapeutists, others by the failure of investigators to recognize the importance of accumulating  $CO_2$  so that many are not admissible as valid evidence of  $O_2$  effects. The legitimacy of admitting data derived from experiments on compressed air as evidence of the effects of increased  $O_2$  alone, may also be questioned, nevertheless even in very recent years compressed air has been employed in experimental studies as a means of providing a desired increase in  $O_2$  concentration, on the assumption that the only variable of physiological significance under compressed air is that of the increased  $O_2$  tension.

Pol and Watelle in a report written in 1847 but not published until 1854, maintained that the rate of breathing was slowed and the thoracic respiratory expansion diminished in men by their exposure to air pressure of about 4 atmos



pheres—i e, an equivalent of about 80 per cent of  $O_2$  at atmospheric pressure Jammet (1871) and Smith (1886) reported that exposure to compressed air in caissons caused an increased frequency of breathing Foley (1863) and Bert (1878) observed that pulmonary capacity was increased in compressed air, but that the respiratory movements were diminished in frequency and magnitude

Kagiyama (1933) found that the frequency of breathing was decreased somewhat by the exposure of his subjects to compressed air at 20 pounds' pressure The results of Ishikawa's (1939) rabbit experiments also suggest that compressed air causes a decrease in respiratory rate, but his observations were made after decompression Shilling, Hansen and Hawkins (1935) reported that the vital capacity expiratory force and the ability to hold the breath were all increased in compressed air

Regnault and Reiset (1849) reported that the respiration of animals was not changed from normal by subjecting them to an atmosphere containing two or three times the concentration of  $O_2$  found in normal air, and Birch (1859) observed that breathing  $O_2$  had no effect on normal men Terray (1897) reported that  $O_2$  in concentrations up to 87 per cent caused no change in breathing in experimental animals

The studies of Speck (1879) made on men led him to support the conclusion of Regnault and Reiset Lukjanow (1884) and Saint-Martin (1884) concluded from experiments on animals and birds that the chemical phenomena of respiration were not altered by breathing hyperoxygenated air, but an examination of the data of each of these latter authors reveals such wide variations as to bring into question the validity of their conclusion

Loewy (1895), administering  $O_2$  in concentrations up to 49 per cent, found the respiratory rate was diminished but that the minute volume was the same as that when breathing ordinary air

Benedict and Higgins (1911) concluded from pneumographic studies on human subjects that breathing  $O_2$  caused "no change in respiration either as to character, depth or frequency, as compared with the same factors when breathing ordinary air "

Davies, Brow and Binger (1925) found that in six subjects the respiratory minute volume when breathing pure  $O_2$  was 2.6 per cent higher than when breathing air, but these authors considered this so near the mean deviation that they were unwilling to lay any stress on this increase Schwab, Fine and Mixer (1936) noted that breathing  $O_2$  (95 per cent) for periods of three hours caused no significant respiratory changes in patients nembutalized for encephalographic studies Anthony (1940) held that breathing  $O_2$  changed neither the respiratory rate nor the minute volume Dautrebande and Haldane (1921) reported that breathing  $O_2$  "increases the breathing" but no data were given as to whether this increase involved respiratory rate, depth, or minute volume, and no differentiation was made between those experiments in which pure  $O_2$  was breathed at atmospheric pressure and those in which it was breathed at the increased barometric pressure of about 2.08 atmospheres

The administration of pure oxygen to anesthetized cats by Wolff and Lennox (1930) caused an increase in the respiratory minute volume Binger, Faulkner

and Moore (1927) found that more prolonged exposures (6 days) of rabbits and dogs to  $O_2$  in concentrations of 70 per cent resulted in respiratory difficulty characterized by a slow rate, increased depth and exaggerated expiratory effort, but this was apparently due to pulmonary complications for there was an accompanying cyanosis and blood stained froth was present in the mouths of the animals. Comparable effects were observed by Paine, Keys and Lynn (1941) in dogs continuously exposed to  $O_2$  in concentrations of 99 to 100 per cent, forty-eight hours of such exposure caused respiratory distress associated with a decline in the  $O_2$  saturation of the arterial blood and death occurred in 60 hours. Such effects might well be considered as those of deficient rather than excess  $O_2$  supply to the tissues.

Richards and Barach (1934) reported that the resting ventilation of human subjects was slightly increased by breathing hyperoxygenated air (40 to 50 per cent  $O_2$ ) but the authors questioned whether the increase might not have been due to a small amount of  $CO_2$  which was present in the chamber during the experiments. Behnke et al (1935) found that the respiratory rate and minute volume of men breathing  $O_2$  remained relatively constant during periods of from eighty minutes to four hours of breathing  $O_2$ , except in two cases, one of whom became dyspneic after two hours and the other developed an increased depth and rate of breathing, and symptoms of bronchial irritation in four hours of exposure. Becker Freyseng and Clamann (1939) became dyspneic within less than two days of continuous exposure to 90 per cent oxygen, but in these experiments, too, there was pronounced pulmonary involvement, no record was made in these experiments or those of Behnke et al of any possible change which may have occurred in the shift from breathing air to the oxygen.

More recently Shock and Soley (1940a, b) have carried out experiments, using a Siebe-Gorman half mask, on thirty three university students. They concluded that breathing  $O_2$  over a period of fifteen minutes induces an increased respiratory minute volume. These authors have suggested three explanations for the increase: 1, a diminution in the amount of oxyhemoglobin reduced with a consequent diminution of available base for  $CO_2$  transport as suggested by Gesell (1923) in the case of breathing  $O_2$  at increased barometric pressure, 2, a reduction in the cerebral blood flow so that there is an increased  $CO_2$  in the respiratory centers, 3, an increased sensitivity of the respiratory center to the normal stimulus. The explanation based on a reduced cerebral blood flow does not appear to be compatible with the finding that  $CO_2$  has so powerful a dilating action on pial vessels as to completely mask any constrictor effect which  $O_2$  of itself might exert (Wolff and Lennox, 1930). Moreover, were it simply a matter of vaso-constriction, one would reasonably expect to find a more highly reduced blood than normal in the veins, actually, however, it has been found (Cusick, Benson and Boothby, 1940) that the retinal vessels, which have been used as an index of what occurs in the deeper vessels of the brain, carry more highly oxygenated blood in their venous ends during  $O_2$  administration than they do when air is breathed, in spite of any vasoconstriction which  $O_2$  may induce in such vessels.

The respiratory rate and minute volume of Hough's (1910) healthy human

subjects were decreased by breathing  $O_2$  in concentrations of from 60 to 80 per cent. An examination of the plotted curves presented by Briggs (1920) indicates that breathing oxygen decreased respiratory rate, in seven of ten subjects the rate of breathing was distinctly lower in  $O_2$  than in air, in two there was no difference and in one the rate was lower in air than in  $O_2$ . The minute volume, however, showed little or no change. A decrease in ventilation during exposure to hyperoxygenated air was also observed in experimental animals by Campbell (1927), and Hamburger et al (1932) concluded from their studies on normal men that both ventilation and vital capacity were decreased by breathing hyperoxygenated air.

Marshall and Rosenfeld (1936) say, "It is well known that in a normal animal or man the breathing of pure oxygen has no effect on the rate or the minute volume of respiration," but in their experimental cats anesthetized with phenobarbital sodium and morphine, they found that  $O_2$  breathing caused a decrease in respiratory minute volume and that this decrease became more pronounced with additional doses of barbital or morphine. Under urethane, chlorbutanol, paraldehyde, or alcohol anesthesia,  $O_2$  produced little or no depression in breathing, dogs responded in a manner similar to that of cats. The authors claim that a prolonged apnea produced by  $O_2$  renders the sinoaortic mechanism less sensitive, so that it no longer is stimulated by anoxemia and that under certain conditions of respiratory depression, the administration of  $O_2$  may further depress respiration or even lead to apnea and respiratory failure. In animals given barbiturates or barbiturates plus morphine, the  $O_2$  depression was found to be severe and easily elicited. The administration of 5 or 7 per cent  $CO_2$  to such  $O_2$  depressed animals frequently failed to stimulate respiration.

Binet and Bochet (1938) and Binet, Bochet and Bryskier (1939) reported that breathing  $O_2$  at atmospheric pressure results in a decrease in respiratory rate. Watt, Dumke and Comroe (1943) found in experiments on seven trained unanesthetized dogs that the inhalation of 100 per cent  $O_2$  at atmospheric pressure through a mask for 6 minutes led to a diminution of from 11 to 31 per cent in the respiratory minute volume within the first minute. In four animals the diminution was transient, a return to normal minute volume having been attained within the 6 minutes, but in three the decrease persisted throughout the period of exposure. This diminution in respiratory minute volume was missing after denervation of the chemo-receptors of the carotid and aortic bodies. The authors concluded that some chemo-receptors in the dog must be continually activated by the usual degree of  $O_2$  unsaturation of the arterial blood at sea level, an observation confirmatory to those of Bernthal (1938), Von Euler et al (1939). In experiments on eleven young human subjects these same authors found that the immediate effect of inhaling 100 per cent  $O_2$  was an increase in respiratory minute volume in thirteen experiments, a decrease in four, and no change in two. The method of  $O_2$  administration and the individual variation among these subjects were not stated, but the authors believed the "findings suggest that tonically active, oxygen-sensitive chemo-receptors are the exception rather than the rule in normal man."

Gesell, Lapides and Levin (1940) found that in administering  $O_2$  in 40 per cent mixtures to dogs the breathing was distinctly less than when breathing 19.7 per cent mixtures and emphasized the nicety of adjustment of reflexogenic breathing to relatively small changes in the  $O_2$  content of the respired air. Keys, Stapp and Violante (1943) found in young healthy men that breathing  $O_2$  (partial pressure of 700 to 715 mm. Hg) at atmospheric pressure through an anesthesia mask (total apparatus dead space 160 cc) for periods of about 17 minutes caused a small increase in ventilation (average +16 per cent, range -4 per cent to +35 per cent) and a small decrease in the minute volume (average -10 per cent, range -20 per cent to -19 per cent).

The lack of unanimity among the various reports concerning the effects of  $O_2$  administration on breathing may, perhaps, be attributable to various influences which make a comparison of the reported effects and an arrival at a final conclusion difficult, if not impossible. Among such influences might be mentioned (1) differences in the duration of the  $O_2$  administrations, (2) failure to record effects of shifting from normal air to  $O_2$  or vice versa, (3) induction of pulmonary complications with attendant alterations in the  $O_2$  saturation of the arterial blood, (4) the techniques used in the  $O_2$  administration, for example, where masks are used the added dead space may account for some differences, particularly since the response to small amounts of  $CO_2$  in air and in  $O_2$  is not always equal, (5) variations in the degree of  $O_2$  saturation of the arterial blood preceding the  $O_2$  administrations—variations which might be due to a variety of causes such, for example, as depth of anesthesia. Taken as a whole, however, the more reliable evidence indicates that the administration of  $O_2$  does cause changes in breathing and in view of the demonstrated tonic response of chemo-receptors to the normal  $O_2$  unsaturation of the blood, it is perhaps only to be expected that  $O_2$  administration might result in changes in breathing. But at best, these changes are not large and may be readily masked by techniques employed in measurement, nevertheless the data appear to indicate that in animal experiments respiratory minute volume is diminished by breathing  $O_2$ , the same may be expected to be true for man but the data, even from more recent investigations, are not unequivocal and for this one may perhaps question to what extent, if any, psychological factors may be responsible.

**Circulation.** The literature contains numerous reports that increased  $O_2$  either in compressed air or in hyperoxygenated air at atmospheric pressure, causes alterations in pulse frequency. A slowing of the pulse rate as a result of exposure to compressed air has been quite consistently observed (Pol and Watelle, 1854, Foley, 1863, Lange, 1864, Vivenot, 1865, Panum, 1868, Bert, 1878, Heller, Mager and Von Schrötter, 1900, Shilling et al., 1936). However, Loewy (1895) and Hill and Greenwood (1906) found no notable alteration in pulse rate as a result of exposure to compressed air, but the latter authors stated that their observations were not sufficiently extensive to permit any final pronouncement.

Demarquay (1866) and Aron (1901) reported that breathing  $O_2$  tended to increase the pulse rate and Wood and Cerna (1890) concluded from two animal experiments that breathing  $O_2$  was without effect on pulse rate, blood pressure or

circulation in general Smith (1870a) presented data which showed that of twelve healthy persons breathing  $O_2$  for short periods the pulse rate was decreased in eight and remained unchanged in the other four "My view," Smith says, "then is, that oxygen acts at the same time upon the heart, to reduce the frequency of its contraction and increase the quantity of blood thrown out at each systole, and upon the blood, to facilitate its flow through the capillaries, that these actions are antagonistic in their effect upon the volume of the pulse and that as one or the other predominates, we shall have the pulse increased or diminished in size, or, if they are exactly apportioned to each other, that there will be no change in volume" Wallian (1889) also concluded that while the effect of breathing  $O_2$  varied to some extent, it almost invariably caused a slowing of the pulse A similar slowing of cardiac frequency by  $O_2$  was observed by Quinquaud (1884) and Loewy (1895) in animal experiments

Benedict and Higgins (1911) in their metabolism studies on men found a very distinct decrease in pulse rate as a result of breathing high concentrations of  $O_2$  (40 to 90 per cent) at atmospheric pressures Convincingly consistent confirmatory evidence that the breathing of  $O_2$  or hyperoxygenated air at atmospheric pressure causes a decrease in pulse rate in man is found in the data and conclusions of numerous later investigators (Parkinson, 1912, Dautrebande and Haldane, 1921, Katz et al, 1932, Behnke et al, 1934, Anthony, 1940, Anthony and Kümmel, 1939, Davis, 1941, Keys, Stapp and Violante, 1943) Schwab, Fine and Mixter (1936) reported that the heart rate of patients nembutalized for encephalographic studies was decreased by breathing 95 per cent  $O_2$  but that this bradycardia persists only for about one-half to one hour after the beginning of the exposure, then disappears Becker-Freyseng and Clamann (1939) found that exposure to 90 per cent  $O_2$  caused a decrease in pulse frequency during the first 15 hours but with continued exposure the pulse frequency increased somewhat, due no doubt to pulmonary complications

Steinhaus, Jenkins and Lunn (1930) experimenting with unanesthetized dogs which had been used for daily metabolism tests for over a period of twenty months found, however, that the administration of  $O_2$  from a Sanborn-Benedict apparatus caused no significant change in pulse rate from that obtaining when breathing ordinary air Binger, Faulkner and Moore (1927) reported that the administration of  $O_2$  in concentrations of about seventy per cent at atmospheric pressure to dogs gave rise to distinct cardiac arrhythmia with extra systoles

Reports concerning the effects of  $O_2$  on blood pressure are less consistent than those on pulse rate Vivenot (1865) held that the magnitude of the radial pulse was decreased in compressed air but that the systolic pressure was increased Bert (1878) recorded an increase in the pulse pressure as well as in both the maximum and minimum blood pressures of animals exposed to air compressed to several atmospheres, the respiratory oscillations in pressure were also increased These changes Bert attributed to the mechanical effects of the increased air pressure rather than to the effects of the increased tension of  $O_2$ , since he found that the administration of  $O_2$  in concentrations of about 35 per cent at atmospheric pressure caused no such changes Jacobson and Lazarus (1877) and Zadek

(1880) reported that the blood pressure increased in compressed air Smith (1886) claimed that the volume of the pulse was always diminished in compressed air Loewy (1895) observed that the blood pressure remained constant or showed a very moderate increase and that the circulation of the blood was not changed from normal in animals under such conditions

Bennett and Smith (1934) reported that exposure of rats to compressed air caused pulmonary hypertension, an affect which was attributed to the increased  $O_2$  tension The recent work of Shilling et al (1936) indicates that both systolic and pulse pressures are decreased in compressed air and that the cardiac output, as calculated from the formula of Furst and Soetbeer (1906), is decreased on an average of about one liter per minute

Wolff and Lennox (1930), in an experimental study on anesthetized cats, found that high concentrations of  $O_2$  at atmospheric pressure lowered both the blood and spinal fluid pressures, the authors pointed out, however, that the  $O_2$  satura

TABLE 1

96 to 99 per cent  $O_2$  inhalation (subject lying on a cot one atmosphere)

SUBJECT	DURATION OF EXPOSURE TO $O_2$	PULSE RATE		BLOOD PRESSURE		
		Start	Finish	Start	Finish	
1	80-240 min	80 Air	59 8 $O_2$	121/77 Air	118/84 $O_2$	Av of 5 readings
2	120-240 min	84 3 Air	68 $O_2$	121/76 Air	119/79 $O_2$	Av of 6 readings
3	120-235 min	69 Air	58 5 $O_2$	124/74 Air	121/86 $O_2$	Av of 4 readings
4	100-240 min.	80 4 Air	66 $O_2$	127/80 Air	120/83 $O_2$	Av of 5 readings
9	125 min.	60 Air	66 $O_2$	120/66 Air	136/66 $O_2$	1 reading subject dyspneic
10	240 min	68 $O_2$	60 $O_2$	128/68 $O_2$	108/80	1 reading bronchial irritation respiratory reaction

tion was slightly subnormal before the  $O_2$  inhalations were begun The administration of  $O_2$  to anesthetized cats for two minutes during constant artificial respiration resulted in a slight rise in blood pressure (Schmidt, 1934) Davis (1941) however found that breathing  $O_2$  caused no significant changes in the blood pressure of anesthetized dogs

Behnke et al (1934) in summarizing their data from experiments on human subjects lying on a cot breathing  $O_2$  in concentrations of from 96 to 99 per cent at atmospheric pressure, state that in all of the  $O_2$  exposures, the blood pressure was constant except in two cases The authors make no specific reference to the alterations induced by the shift from air to  $O_2$  but an examination of their tabulated data from six subjects reveals that the average systolic blood pressure was decreased and that the diastolic pressure was increased by breathing  $O_2$  in all except one subject, in the exception, who had become dyspneic, the systolic pressure was increased by breathing  $O_2$  and the diastolic pressure remained the same as when breathing air A compilation of averages made from the data of Behnke

et al is shown in table 1 above. Such data would seem to support a conclusion that systolic pressure is decreased, diastolic pressure slightly but distinctly increased with a consequent drop in pulse pressure, and that the pulse rate is definitely diminished, by breathing  $O_2$ .

Keys, Stapp and Violante (1943) reported that in young men breathing almost pure  $O_2$  at atmospheric pressure caused a slight but consistent rise (4 mm Hg) in diastolic blood pressure and a tendency to an increased systolic pressure, the pulse pressure was somewhat decreased. There was a slight decrease in cardiac "work" and "effort" but no significant change was observed in either cardiac size (roentgenkymograph) or in cardiac efficiency.

Katz et al (1932) noted in their study of  $O_2$  therapy for cardiac cases that neither the arterial nor venous blood pressure, of their control subjects (human) was altered significantly by breathing hyperoxygenated air but the T wave of the E K G was lengthened, the change was not explainable on the basis of any possible relief of arterial anoxemia. Schwab, Fine and Mixter (1936) observed no significant change in blood pressure of nembutalized patients as a result of their exposure to 95 per cent  $O_2$  for three hours. Anthony (1940) reported that breathing  $O_2$  caused a decrease in stroke volume of the heart and changes in the E K G. Richards and Barach (1934) found that the cardiac output of normal men was not changed by their residence in hyperoxygenated air (45 per cent  $O_2$ ) at atmospheric pressure.

A perusal of the evidence leaves little doubt but that breathing  $O_2$  or hyperoxygenated air at atmospheric pressure causes a predominant slowing of the heart. The reports concerning blood pressure changes are not unequivocal, but pulse pressure is seemingly diminished.

*Vascular Changes* The early reports to the effect that breathing hyperoxygenated air at atmospheric pressure or compressed air caused peripheral vascular changes were based for the most part upon observation of changes in skin colour of individuals during their exposure to compressed air and in some cases even after their decompression to normal pressure. Such changes were not infrequently attributed to the supposed direct mechanical effect of the pressure itself.

The experiments of Poiseuille (1835) in which he demonstrated by direct observation that the peripheral capillaries of frogs, tadpoles and salamanders were not altered by the animals' exposure to compressed air should have dismissed the notion that either the pressure itself or the attendant increase in  $O_2$  tension, caused any distinct constriction of the peripheral vessels. But in spite of this work, the contention of Bert (1878), and the observation of Hunter (1887) that no constriction occurred in the fundic vessels of men on their exposure to compressed air, the idea that in compressed air the increased pressure *per se* caused a peripheral vasoconstriction was held and argued by numerous investigators, typified by Vivenot (1865) and Smith (1870, 1886) even to the early 1900s.

Hill and Macleod (1902), unaware of Poiseuille's experiments and feeling the need of further experimental evidence on the question of a peripheral vascular constriction, exposed frogs to high pressures of air by a technique similar to that of Poiseuille and found no alteration in capillary circulation even at pressures as

high as 70 atmospheres. Thus the findings of Poiseuille were abundantly confirmed and the interpretation that increased pressure *per se* or increased  $O_2$  tension in the compressed air, was shown to be untenable.

L. Smith (1899) had suggested that the lung changes he observed in animals which had been exposed to high  $O_2$  pressures might represent an attempt on the part of the organism to protect the tissues against excessively high  $O_2$  tensions. Following this suggestion of a protective reaction it was only natural that attempts would be made to look for other protective mechanisms, and what more convenient or purposive mechanism could there possibly be imagined than one which closed off the  $O_2$  supply to the tissues by constricting the blood vessels?

Dautrebande and Haldane (1921) performed experiments which, as they said, "were undertaken for the purpose of seeing whether any evidence could be obtained that the tissues of the nervous system are defended against the influence of oxygen by diminution of the circulation through them." The conclusions drawn from their experiments have been accepted so frequently as substantial evidence of a vasoconstriction induced by  $O_2$  that the argument of the authors is presented here.

"If the circulation is diminished when oxygen is breathed," they said, "it is evident that the pressure of  $CO_2$  in the tissue must rise, and in the respiratory centre this rise of  $CO_2$  pressure will, other things being equal, imply rise of hydrogen ion concentration and consequent increase in breathing and fall of alveolar  $CO_2$ -pressure. A very slight fall in alveolar  $CO_2$ -pressure will, however, suffice to compensate for the rise in  $CO_2$ -pressure in the tissues in consequence of a sufficient slowing down of the circulation to reduce the oxygen pressure of the venous blood to normal. Complete compensation could not, however, be expected, since otherwise there would be no stimulus left to account for the slowing down of circulation."

"The problem which we set ourselves to investigate, therefore, was whether there is any fall in alveolar  $CO_2$ -pressure when oxygen at increased partial pressure is breathed. We also watched the pulse carefully, as any diminution in pulse-rate would serve as an index of slowing of the circulation." These investigators did find a drop in the alveolar  $CO_2$  partial pressure of about 1.5 mm Hg and a slowing of the pulse when  $O_2$  was breathed at normal barometric pressure, and remarked, "The experiments therefore confirm the theory (which is in itself probable from many considerations into which we need not enter here) that the excess of free  $O_2$  in the arterial blood causes slowing of the circulation." The validity of this argument and the supposed evidence of vasoconstriction are obviously open to very serious question, especially since there was also an increase in breathing which of itself would lower alveolar  $CO_2$  tension.

Retzlaff (1913) concluded from plethysmographic studies that  $O_2$  caused a vasoconstriction in the lungs. Faulkner and Binger (1927) reported that the capillaries in frogs were not noticeably affected by the administration of  $O_2$  in concentrations as high as 95 per cent.

Tinel (1927) observed a dilatation in the exposed superficial vessels of the brain of cats on the cessation of  $O_2$  inhalation. Rebreathing from a bag of  $O_2$



did not produce vasoconstriction, on the contrary a vasodilatation (probably due to accumulated  $\text{CO}_2$ ) was observed. From these findings Tinel suggested that  $\text{O}_2$  caused vasoconstriction and that the cerebral circulation may be regulated by the  $\text{O}_2$  content of the blood. Campbell and Hill (1931) suggested that Tinel's claim might be put to the test by an observation of the retinal vessels, Duke-Elder (1931) making such an examination found no change from normal in the vessel calibre when his subjects breathed  $\text{O}_2$  for periods of five minutes.

Wolff and Lennox (1930) in studies on exposed pial vessels of cats observed that an increase in the  $\text{O}_2$  tension caused a slight vasoconstriction, and the addition of small amounts of  $\text{CO}_2$  to the respired gas resulted in pronounced dilatation which masked the distinctly lesser tendency for constriction by  $\text{O}_2$ . The authors were reluctant to accept their results as indicative of similar cerebral circulatory influence of  $\text{O}_2$  on man for two reasons: 1, because their experimental animals were under amytal anesthesia, 2, because the vessels in the pial covering, they thought, may act differently than the vessels in the brain substance. They further cautiously pointed out that the changes they observed may have been related to the sub-normal arterial  $\text{O}_2$  saturation (75 per cent) obtaining before the  $\text{O}_2$  inhalation (due, perhaps, to the anesthesia) and a rise to 95 per cent saturation on the inhalation of the  $\text{O}_2$ . They maintained that it does not necessarily follow that a similar increase in arterial  $\text{O}_2$  above normal would give a comparable vascular change.

In studies relating blood flow and inhalation of various  $\text{O}_2$ - $\text{CO}_2$  mixtures by human subjects, Lennox and Gibbs (1932) found that when breathing pure  $\text{O}_2$  the  $\text{O}_2$  content of the arterial blood increased by 1.2 volumes per cent. The changes in blood flow, they found, were comparatively slight and inconsistent. "The results," they say, "therefore are indecisive, there being evidence of a slight decrease in the speed of blood flow through the brain and more consistent evidence of an increased flow through the leg." The same authors found that an increased  $\text{CO}_2$  tension of the arterial blood caused an increased flow through the brain and a decrease through the leg. The reciprocal allotment of blood flow through the brain and through the peripheral tissue during  $\text{O}_2$  inhalation would appear then to be just the reverse of that induced by  $\text{CO}_2$  administration, excess  $\text{O}_2$  seems to favor, though to small degree, an increase of blood flow to the periphery while excess  $\text{CO}_2$  favors a shift of flow to the brain.

This observation on the reciprocal adjustment to  $\text{CO}_2$  confirms the findings of Gesell and Bronk (1926) that the administration of  $\text{CO}_2$  caused an increased blood flow through the carotid artery and to the brain. Bronk and Gesell (1927) and Bernthal, Bronk, Cordero and Gesell (1928) not only showed that both low  $\text{O}_2$  and  $\text{CO}_2$  increased the volume flow of blood through the carotid artery, but that there was a parallel increase in the blood flow through the vertebral artery, thus demonstrating that carotid blood flow is a safe index of the blood flow through the brain. The breathing of pure  $\text{O}_2$  at atmospheric pressure, or  $\text{O}_2$  in 50 per cent mixtures, caused a decrease in the carotid and femoral volume flow of blood.

Kroetz (1930) deduced from experiments on normal human subjects that

breathing  $O_2$  at increased concentrations caused a large increase in the volume flow of blood and an increase in the  $O_2$  tension of the tissues

The view that  $O_2$  causes a vasoconstriction in the brain finds some support, however, in the experiments of Schmidt (1934) on the regulation of the circulation in the hypothalamus of the cat. He observed that the administration of  $O_2$  occasionally gave a slight vasoconstriction as indicated by a thermo-junction thrust into that tissue. Dumke and Schmidt (1943) measured the cerebral blood flow through the basilar and internal carotid arteries in nembutalized Macaque monkeys and found "there was a distinct hint of a constrictor action" when breathing oxygen (100 per cent), but this effect was never at all marked, a dilating effect of  $CO_2$  (10 per cent) was consistently observed. When  $CO_2$  is administered along with increased  $O_2$  tensions any vasoconstrictive influence which  $O_2$  may exert on pial or retinal vessels is nullified by the dilating action of the  $CO_2$ , the result is an increased blood flow to the brain (Wolff and Lennox, 1930, Cobb and Fremont-Smith, 1931, Cusick et al. 1940).

Cusick, Benson and Boothby (1940) found that breathing pure  $O_2$  at atmospheric pressure for 30 minutes, decreased the calibre of both arterioles and veins in human retinæ by from 10.5 to 37.7 per cent, usually this change was more marked in the veins. It was also observed that in spite of any constriction which occurred when breathing  $O_2$ , the venous blood was much redder than when breathing air. Similarly Cobb and Fremont-Smith (1931) observed in men that the colour of the retinal veins became arterial when  $CO_2$ - $O_2$  was breathed.

Rosenthal (1939) reported that in fifteen human subjects the inhalation of  $O_2$  caused a narrowing of physiological angioscotomas, after the withdrawal of the  $O_2$  there occurred a widening of the scotomas to areas in excess of that preceding the  $O_2$  administration. The pattern of the narrowing induced by  $O_2$  inhalation followed that of the retinal vessels which suggested the effect was of peripheral rather than of central action of the  $O_2$ , the retinal synapse was proposed as the probable site of this influence. Cusick et al. (1940) interpret Rosenthal's findings as due to the involvement of an  $O_2$  vasoconstriction.

An evaluation of the evidence concerning the influence of  $O_2$  on vessel calibre supports the conclusion that  $O_2$  at atmospheric pressure does cause a slight vasoconstriction in the C.N.S. and retinæ. But the fact that the venous blood is not normally reduced when breathing  $O_2$  indicates that if this vasoconstriction is a reaction on the part of the organism to protect its tissues against an excessively high arterial  $O_2$  tension, it is grossly inadequate as a protective mechanism. The finding that a relatively small increase in  $CO_2$  completely masks any vasoconstrictive influence of  $O_2$  is important, not only in the interpretation of vasomotor responses to breathing  $O_2$  at atmospheric pressure, but also and especially in the interpretation of the effects of  $O_2$  at pressures above one atmosphere. In any case a localized vasoconstriction cannot be regarded as an infallible index of a generalized vasoconstriction,—a point well emphasized by Wiggers (1942) in connection with his studies on shock.

**Blood.** In recent years a renewed interest in the blood changes induced by breathing  $O_2$  rich mixtures has become manifest. This interest has been for the

most part concerned with the changes in cell counts, cell size, and hemoglobin Heller, Mager and von Schrötter (1900) observed no significant change in the R B C 's of caisson workers as a result of exposure to the increased air pressure, but Bornstein (1911) found that exposure of a dog to air pressure of about two atmospheres from October to the following April caused a decrease in the R B C count and a drop in hemoglobin from 100 to 85 per cent, these and similar changes found in growing dogs and a monkey, but not in pigeons, were attributed to an increase in the blood volume Ishikawa (1939) likewise reported that exposure of dogs to compressed air (80 85 or 130 lbs ) caused a decrease in hemoglobin content of the blood

Karsner (1916) after having made careful studies on twelve control animals in which he found considerable variation in the R B C counts (between 8,460,000 and 5,512,000 per cm ) stated that prolonged exposure to high concentrations of O<sub>2</sub> (80 per cent to 90 per cent) at atmospheric pressure, appeared to produce no material changes in the erythrocyte count that were not observed in control animals living for similar periods under the same general conditions The variation in the per cent of reticulated R B C 's of the control animals was such (2 to 25 per cent) that no very definite conclusion could be made concerning the action of O<sub>2</sub> on this type of cell Likewise, the resistance of the R B C 's to hypotonic solutions, and the hemoglobin percentage as determined by the Talquist scale, showed no distinct changes

In four out of five of Karsner's experimental animals there was a consistent and appreciable leucocytosis as a result of the O<sub>2</sub> exposure (the fifth animal was not acceptable because of an abnormally high count before exposure) This increase in leucocytes was apparently unrelated to the occurrence of pneumonia observed in the O<sub>2</sub> exposed animals, Karsner believed it was "probably to be explained only as accidental variations so frequently seen in the rabbit " There was a pronounced phagocytosis of the R B C 's in the lymph nodes of the O<sub>2</sub> exposed animals and this was thought to be associated with the passive congestion found in other organs The bone marrow of ten animals exposed to O<sub>2</sub> showed no distinct departure from the picture seen in the controls The clotting time was likewise unaffected by the O<sub>2</sub> exposures

Full and von Friedrich (1923) subjected healthy men, diabetics, and hemiplegic patients to O<sub>2</sub> at pressures of +10 to +8 cm of H<sub>2</sub>O by means of a gas mask and found there was a significant decrease in hemoglobin content of the blood They concluded that this change was caused by a movement of tissue fluid, low in albumin, sugar, and NaCl, into the blood stream Izumiyama (1928) in somewhat similar experiments found that breathing O<sub>2</sub> at both atmospheric and at slightly increased pressures (+8 to +20 cm H<sub>2</sub>O) caused a decrease in both hemoglobin content (10 to 20 per cent) and in the R B C count However, since the mechanical effects of an unequalized positive pressure may cause reflex and direct circulatory changes, it is questionable whether the results of these experiments may be safely interpreted simply as O<sub>2</sub> effects

Barcroft, Hunt and Dufton (1920) studying the treatment of chronic gas poisoning by residence in a chamber containing O<sub>2</sub> in 50 per cent concentrations,

found that when the R.B C count before the treatment was greater than five million the treatment reduced the count, but when it was less than five million subsequent residence in the O<sub>2</sub> chamber did not alter it

Campbell (1927a b) concluded that exposures to hyperoxygenated air at atmospheric pressure (O<sub>2</sub> content as high as 200 per cent above normal), for 3 or 4 weeks caused a decrease in both hemoglobin percentage and in the R.B C count in rabbits, rats, mice, "cavies", and monkeys (cats were not so affected, but the color index increased) These results suggested that Bornstein's earlier finding of a decreased R.B C count in compressed air was due to the increased O<sub>2</sub> tension, but Campbell disagreed with Bornstein's interpretation that such change was caused by a hemodilution, because of the lack of parallelism between changes in hemoglobin and R.B C count Although the color index increased in Campbell's animals during the exposure to the increased O<sub>2</sub> to a point resembling that observed in pernicious anemia, neither the size nor the type of R.B C was altered

In the experiment in which the reticulated R.B C 's were examined, Campbell found 8 per 1000 at the end of a four weeks' exposure to the increased O<sub>2</sub> tension, whereas ten days after return to normal air they had increased to 80 per 1000 This suggested that the high O<sub>2</sub> concentration decreased the formation of R.B C 's, but in view of the fact that this represents data from only one experiment, that apparently no pre-exposure estimation was made, and that very wide variations in reticulated cells occur even under ordinary conditions (Karsner, 1916), perhaps the results are less significant than they might at first appear The changes in the white cells were not sufficiently consistent to be significant, but the differential counts showed the lymphocytes were appreciably increased by the O<sub>2</sub> exposure

Achard, Binet and LeBlanc (1927) found an increase in both the R.B C and leucocyte counts in their experimental animals (guinea pigs and rabbits) after 48 hours of exposure to 80 per cent O<sub>2</sub> at atmospheric pressure with a partial return to normal at the end of 120 hours of exposure It was later reported (Binet and Bochet, 1938, Binet, Bochet and Bryskier, 1939) that breathing hyperoxygenated air caused an initial decrease followed by an increase in the R.B C count to values as high as 150 per cent above normal It was also noted (Binet, Bochet and Guiraud, 1939) that while 70 per cent O<sub>2</sub> mixture caused decrease in the R.B C count of guinea pigs and rabbits within the first few hours of exposure, it might still be subnormal after several days Very similar initial results were observed in guinea pigs with 40 per cent O<sub>2</sub> mixtures and in men breathing 60 per cent O<sub>2</sub> from oxygen tents The results reported by these investigators suggest that the lack of unanimity of opinion among various investigators as to the effects of increased O<sub>2</sub> concentrations on the R.B C counts, may perhaps be explained by differences in the time at which the counts were made and in the concentrations of the O<sub>2</sub> employed

Anthony and Bechthold (1939) noted that in men even the first few minutes of breathing O<sub>2</sub> caused a drop in the hemoglobin content of the blood and a decrease in the R.B C count These changes were only temporary, however, for within fifteen minutes recovery had begun and after fifty minutes the original values

had been regained. The decrease in hemoglobin and R B C count was attributed to an increased blood volume resulting from a shift in tissue fluid (Anthony, 1940). Paine, Keys and Lynn (1941) reported a marked increase in the hemoglobin (associated with a decline in  $O_2$  saturation of the arterial blood) of dogs exposed to 90 per cent  $O_2$  for several days. Davis (1941) found a slight but constant increase in both the hemoglobin content and in the R B C's as a result of administering  $O_2$  (1000 cc per min) by nasal catheter to anesthetized dogs, but the W B C's were reported to be definitely decreased by as much as 10 to 30 per cent of their initial value. Behnke et al (1934) found an increased leucocyte count in three men after four hours of breathing  $O_2$  at one atmosphere pressure. Becker-Freyseng and Clamann (1939) observed a very distinct increase in the leucocytes (from 6,200 to 12,700) but only a very slight increase in R B C's in themselves as a result of their exposure to  $O_2$  in concentrations of about 90 per cent.

The size of the blood cells has also been found by various observers to be altered by breathing increased concentrations of  $O_2$ . Mannasein (1872) reported that the diameter of the R B C's was increased by breathing  $O_2$ . Gunther (1928) observed that breathing  $O_2$  caused a decrease in R B C diameter from  $7.14 \mu$  to  $6.72 \mu$ . Similarly Hitzenger and Molenaar (1934) reported that in healthy persons the breathing of  $O_2$  for twenty minutes caused a decrease in both the diameter (from  $7.3 \mu$  to  $7.1 \mu$ ) and the volume of the R B C's as well as a decrease in serum albumin and an increase in blood volume. The dilution of the blood was attributed to tissue fluids flowing into the vessels from plasma depots and a release of water from the R B C's. The authors think that prolonged changes in the  $O_2$  tension in the air breathed—even that associated with the usual changes in barometer reading—may cause a distinct effect on the blood and its constituents.

Schmidt-Lange and Podloucky (1937) likewise found that in mice, guinea pigs, and rabbits the diameter of the R B C's was decreased by breathing  $O_2$  and thought this was due to a disturbance of the  $O_2$ - $CO_2$  relation and the acid base equilibrium of the body. All of the ten healthy persons studied by Anthony and Bechthold (1939) showed an average decrease in R B C diameter of 2.1 per cent as a result of breathing  $O_2$ . This was attributed to an increased alkali binding ability of the oxyhemoglobin. Taken as a whole the reports on cell size indicate that breathing  $O_2$  or hyperoxygenated air caused a decrease in R B C diameter.

The reports of chemical changes occurring in the blood as a result of exposure to increased concentrations of  $O_2$  have not been very extensive. That there is an increase in the amount of  $O_2$  in solution has, of course, long been recognized, even before the time of Bert. Quinquaud (1884) found that breathing pure  $O_2$  increased the  $O_2$  content of the arterial blood 2.2 volumes per cent. Hough (1910) explained the changes in breathing which he observed in  $O_2$  at concentrations of 60 to 80 per cent as due to an increase of 1.2 volumes per cent of  $O_2$  dissolved in the blood of his subjects. Hill (1912) gives the  $O_2$  solubility of the blood at body temperature as 2.4 volumes per cent. This would mean, assuming complete

equilibrium in the lungs, that the  $O_2$  carriage by the blood to the tissues would be increased by more than 10 per cent if the alveoli were filled with  $O_2$ . Lennox and Gibbs (1932) found that the  $O_2$  content of arterial blood in man was increased 1.2 volumes per cent by breathing pure  $O_2$ , but that the  $CO_2$  content was not appreciably altered. Analyses made by Binger, Faulkner and Moore (1927) showed that in dogs after several days' exposure to hyperoxygenated air (70 per cent  $O_2$ ), the arterial blood was only 40 per cent saturated. Such a finding is particularly significant in relation to  $O_2$  therapy, it indicates that while breathing  $O_2$  may initially increase the  $O_2$  content of the blood, its continuous administration to a point where lung damage is induced, may actually result in subnormal  $O_2$  content of the blood.

Ishikawa (1939) reported that exposure of dogs to air pressures of from 60 to 130 pounds caused a decrease in the blood lactic acid and an increase in blood sugar. Davis (1941) found that  $O_2$  administration to dogs caused no significant change in blood sugar, chlorides or N P N, but that there was a decrease in  $CO_2$  combining power of the blood which persisted for as long as twenty minutes after cessation of the  $O_2$  administration. Mention has already been made of reports on changes in blood urea and  $CO_2$  (see section on metabolism).

*Influence of  $O_2$  on Enzymes* Investigations carried out in recent years provide ample evidence that increased concentration of  $O_2$  at atmospheric pressure inhibits enzyme activity. That this should be so is perhaps not so surprising in view of the inhibitory effects of increased  $O_2$  tensions in some inorganic reactions. Brooks (1942) points out that  $O_2$  is an inhibitor of many inorganic thermal and photochemical reactions and his own experimental data suggested that in the oxidation of hemoglobin,  $O_2$  acts both as a reactant and inhibitor as it does in the case of irradiated quinine (Weigert, 1912).

Laqueur (1912) reported that the rate of proteolysis in liver tissue was slowed by  $O_2$ . McCance (1924), studying autolysis, found that urea formation was inhibited by  $O_2$ . Voegtlin and Maver (1932) and Maver, Johnson and Voegtlin (1933) showed that a decrease in  $O_2$  tension accelerated proteolysis. Lee and Chen (1938) reported similar results from experiments on acid glycerol extract from sheep liver and that this inactivation of proteolytic enzymes was only partially reversible. Libbrecht and Massart (1934) also found proteolytic enzymes were inhibited by  $O_2$ . The experiments of Bailey et al (1942) likewise show an inhibitory action of  $O_2$  on proteolytic enzymes. Albaum, Donnelly and Korkes (1942) working with oat seedlings found suggestive evidence that increased  $O_2$  tension interferes with proteolysis. Rondoni and Pozzi (1933) however reported the rate of proteolysis in liver extract was not altered by  $O_2$ .

• Marks and Fox (1933) and Marks (1935a, b) found that catalase from some species of marine plants and 19 marine animals were inactivated either directly or indirectly by  $O_2$  and enzyme assays of Albaum et al (1942) carried out on oat seedlings thirty hours after the grain had been soaked in oxygenated solution showed that such oxygenation decreased catalase activity.

Albaum et al. (1942) also found that oxygenation decreased the endogenous dehydrogenase activity of oat seedlings, and Shapiro and Wertheimer (1943) re-

ported that the  $O_2$  in room air readily inhibited the activity of fatty acid dehydrogenase in mixtures with coenzyme and substrate. Formic dehydrogenase of *B. coli* has also been found (Gale, 1943) to be inactivated by increased  $O_2$  tension. Lehmann (1935) reported that the activity of succino-dehydrogenase had its optimum  $O_2$  tension at from 44 to 56 mm Hg, at higher tensions it was very distinctly lowered. This toxic effect of increased  $O_2$  tension was said to be irreversible and the critical pH value for this effect lay in the neighborhood of 7.4. In studies on *Acetobacter peroxydans*, Weiland and Pistor (1938) found lactic acid dehydrogenation was inhibited by  $O_2$ .

In experiments on red blood cells (human) and tissue slices, Jowett and Quastel (1933a, b) found that glyoxalase activity was diminished by  $O_2$ , that this inhibitory effect increased as the exposure was continued, and that it was a reversible process. The authors indicated that this effect of  $O_2$  is probably due to a partial oxidation of glutathione which results in a decreased co-enzyme concentration and thus of enzyme activity.

Hellerman, Perkins and Clark (1933) and Hellerman (1937) also report that  $O_2$  has an inhibitory influence on many enzyme systems and emphasize the importance of the presence of heavy metal ions in some of these inhibitory reactions. It was found that several hours' aeration of highly purified, crystalline urease reduced the activity by only about 10 per cent, but in the presence of cupric ions the ureolytic activity was completely abolished by aeration for only one-half hour and irreversibly destroyed by aeration for 3.5 hours. These results were interpreted to indicate that the "poisoning" effects of cupric ions were not due to the ions themselves but rather to their ability to accelerate the oxygenation of urease. This influence of the ions of heavy metals such as iron, and especially copper, was also pointed out to be operative in the case of cysteine which, in highly purified form, was reported to be but slowly attacked by  $O_2$  but when in the presence of ions of iron or copper it, as was true also for other thiol compounds, was rapidly oxidized.

*Pathology* The first suggestion that breathing  $O_2$  caused pathological effects seems to have come from the observations of Priestley. Lavoisier (1783) was aware of these observations and having, as he said, occasion to repeat some of Priestley's experiments chose guinea pigs for the purpose. Autopsies were performed on these animals which had died in "vital air" and he found that in every instance death appeared to have been caused by "une fièvre ardente" and a "maladie inflammatoire." "The flesh was a *very red* colour, the heart livid, and turgid with blood, especially the right auricle and ventricle, the lungs were very flaccid, but *very red*, even externally", they were also engorged with blood.

Beddoes, one of the earliest advocates of the therapeutic use of  $O_2$ , recognized that the injudicious use of  $O_2$  might cause pulmonary damage. He says, "It is not then defect but excess of Oxygene that is pernicious here. The heart and arteries pulsate more quickly and forcibly, the eyes grow red and seem to protrude, the heat of the body is said to considerably increase (a), sweat to break out over the whole body and fatal mortification of the lungs to come on. These appearances denote violent inflammation. The production of inflammation is fully

established by dissection" (Beddoes and Watt, 1796) An experiment was performed on a large kitten which was kept for seventeen hours in an atmosphere of 80 per cent  $O_2$ . At autopsy it was found that the lungs were a florid red color. The edge of one lobe "was marked with livid spots (as in mortification). The pleura was likewise evidently inflamed", the lungs of the control kitten were "pale."

The first report of any extended experiments on the action of  $O_2$  in successive exposure appears to be that of Dumas (1866) who, thinking  $O_2$  might have some "baleful" action on some types of phthisis, tested his theory on a dog. A healthy animal was placed in a large chamber in which the air had been replaced by  $O_2$  and which was fitted with inlet and outlet tube for maintaining a constant purity of the gas. The animal was kept in this atmosphere for a period of six hours, at the end of which time its respiration appeared to become "more precipitate, more rapid", the animal was then moved to ordinary air. This same procedure was repeated twice a day for 28 days, at which time, because of manifest respiratory difficulty, the periods of  $O_2$  exposure were shortened but continued for 15 days longer. At that time the dog's breathing had become "sonorous, slow, and laborious," his weight had fallen, and he became emaciated. The animal was killed, and its lungs examined, the right side of the thorax was found filled with "acid serum and coagulated blood, the bronchial tubes were filled with fluid, the pleura was tightly adherent to the lungs especially at the bases which were adherent to the adjacent parts. The membrane was red, tumefied and as if affected by inflammation. The lungs, red and seamed with little rents, had become considerably solidified as occurs in the case of organs which have been for some time inflamed." In the vicinity of the bronchial tubes there was found a separating abscess. Dumas says, "The anatomical inspection of these organs, therefore, did not permit one to doubt that  $O_2$  had induced an irritating action upon the lungs and that from it had resulted all the ordinary symptoms of phthisis." Demar quay (1866), another ardent  $O_2$  therapist, recognized a danger in the use of  $O_2$  and maintained that particularly in the presence of a tendency to inflammation this gas may give rise to accidents.

Although the data from these early experiments can be accepted only with some degree of reservation due to the small number of experimental animals employed and the question of adequate controls, they do find support in later and more carefully controlled investigations.

Moir (1895) reported that mules which had been continuously exposed for several months to compressed air at about 30 pounds' pressure in the Hudson Tunnel showed no adverse effects. This was taken as evidence that 60 per cent concentrations equivalent to 60 per cent of an atmosphere is tolerable. Smith (1899) found pronounced pulmonary pathology in mice, birds, and guinea pigs and pigeons which had been exposed to  $O_2$  at atmospheric pressure. The lungs were filled with a "granular" exudate, no leucocytes were present. The condition resembled the "earliest stages of croupous pneumonia." The pulmonary "inflammation," congestion, and consolidation, in addition to the congestion of other organs, particularly the liver, spleen and heart, was also



thology was essentially the same in both mammals and birds. The higher the  $O_2$  tension the earlier was the onset of the inflammatory reaction, but  $O_2$  at tensions equivalent to 40 per cent of an atmosphere was without effect and failed to produce pneumonia in mice which had been exposed to it for as long as eight days. In  $O_2$  concentrations of about 80 per cent, however, some mice died after only four days of exposure. Smith pointed out that the autopsies of individuals dying of caisson sickness had sufficient features in common with  $O_2$  poisoning to suggest that the increased  $O_2$  tension in compressed air may contribute to the pathology of caisson sickness.

Hill and Macleod (1903c) observed that  $O_2$  at atmospheric pressure did not cause any symptoms of pneumonia in mice in a 6 hour exposure. Air at a pressure of 5 atmospheres for periods up to as long as 24 hours likewise caused no symptoms of pneumonia. It was believed that such a duration was too short to induce any distinct lung pathology. But at air pressures of 7 atmospheres there occurred gasping and death and at autopsy extensive pathology resembling that described by Smith (1899) was found in the lungs. The authors denied, however, that the increased  $O_2$  tension in the air pressures commonly employed at that time was a contributory factor in the occurrence of caisson sickness as Smith had claimed.

Schmeidehausen (1909), using mice, rabbits and guinea pigs in experiments in which some of the animals were exposed to pure  $O_2$  at atmospheric pressure in a chamber and others breathed  $O_2$  through a tracheal cannula, found pathological changes in the lungs characterized by hyperemia, edema, atelectasis and inflammatory processes, but no *true* pneumonia occurred in exposures of less than 69 hours. David (1912) demonstrated changes essentially similar to those of Schmeidehausen. Schmidt and David (1911, 1912) emphasized the danger in the prolonged use of  $O_2$  in chloroform anesthesia and claimed that even in concentrations as low as 40 to 60 per cent,  $O_2$  by itself induces inflammatory changes in the lungs if the exposure be prolonged to 70 hours.

Bornstein and Stroink (1912) found that continuous breathing of  $O_2$  in concentrations as low as 60 per cent for several months produced anemia in a dog and monkey and that at somewhat higher percentages, desquamation, edema and inflammation with some hemorrhage occurred. Brüning (1912), however, claimed the changes described by these two authors were really caused by the dryness of the gas rather than by the  $O_2$  itself, to which charge Bornstein (1912) replied in defense of the evidence of Bornstein and Stroink.

Adams (1912), experimenting with guinea pigs, found that breathing  $O_2$  in concentrations of about 80 per cent caused death in from three to four days. Post mortem examination showed the lungs to be markedly congested, all the lobes were distended, the heart was engorged as in asphyxial death, exudate was present in the alveoli and the epithelium showed desquamation, bacterial smears and cultures made from the cut lung were negative. Death was attributed to an acute "lobar or catarrhal pneumonia." It was concluded that  $O_2$  in concentrations below 70 per cent can be inhaled for prolonged periods without giving any symptoms of pathology, but in higher concentrations its use is attended with serious risk of causing an irritative pneumonia and death.

Karsner (1916), emphasizing the need of further studies on the possible pathology induced by breathing atmospheres rich in  $O_2$ , carried out investigations dealing with the effects of  $O_2$  in 80 to 96 per cent mixtures at atmospheric pressure. Great caution was observed in the matter of controls since autopsies had frequently revealed the presence of significant pathology in supposedly normal, non-symptomatic laboratory animals—especially rabbits.

Among the changes induced by increased concentrations of  $O_2$  Karsner found dilatation of either the right or both sides of the heart in animals which had succumbed to, or survived, 2 to 5 days of exposure to the increased  $O_2$ , cloudy swelling became practically uniform in the longer exposures. The aorta showed no notable change. The liver was passively congested, and this, it was thought, might or might not have been associated with hemosiderin pigmentation, but it was not prominent until after 72 hours of exposure when it was associated with intercellular edema, the stomach and intestines were also congested.

In the kidneys too the most prominent change was one of congestion, cloudy swelling appeared to be more evident in the kidneys of high  $O_2$  animals than in the controls, but Karsner questioned if the difference was great enough to be of significance. Albuminuria appeared in two of the  $O_2$  exposed rabbits, six positive cases of interstitial nephritis which occurred were explained as having probably arisen from the superimposition of passive congestion on kidneys already diseased, but diseased so that under ordinary conditions albuminuria did not exist. The adrenals and spleen showed no significant alteration other than that of congestion. Lymph nodes appeared to indicate a more marked phagocytosis of R.B.C.'s associated with the passive congestion. Bone marrow from ten exposed animals showed no distinct difference from that of the controls and the blood was not thought to be significantly altered.

Karsner, like Hill and Macleod (1903c), was impressed by the wide individual variation in susceptibility to the effects of  $O_2$  poisoning. He suggested that the susceptibility to the deleterious effects of  $O_2$  might be accentuated by living through the diseases which caused the non-experimental lesions—a point of considerable interest, especially to those  $O_2$  therapists who have maintained that a damaged lung is less susceptible to any injurious effect of  $O_2$  than a normal lung. Karsner's finding of fibrin in the inflammatory reactions of the lungs is contrary to that of Smith (1899), but both of these investigators reported that the leucocytes were not significantly altered. The passive congestion in the lungs and all abdominal viscera and which was accompanied by "secondary changes such as cloudy swelling of the parenchymatous organs and phagocytosis of erythrocytes by endothelial cells of the mesenteric lymph nodes" was attributed by Karsner to the failure of the heart, either as a whole or its right side. It was concluded that "atmospheres containing 80 to 96 per cent oxygen under normal barometric pressure produce in 24 hours, or more commonly 48 hours, congestion, edema, epithelial degeneration and desquamation, fibrin formation, and finally a pneumonia, probably of irritative origin and to be described as a fibrinous bronchopneumonia." Further work by Karsner and Ash (1916) confirmed these findings and also showed that while 53 per cent  $O_2$  caused no change in rabbit lungs in exposures of about 3.5 days, 67 per cent induced slight evidence of pneumonia but

thology was essentially the same in both mammals and birds. The higher the  $O_2$  tension the earlier was the onset of the inflammatory reaction, but  $O_2$  at tensions equivalent to 40 per cent of an atmosphere was without effect and failed to produce pneumonia in mice which had been exposed to it for as long as eight days. In  $O_2$  concentrations of about 80 per cent, however, some mice died after only four days of exposure. Smith pointed out that the autopsies of individuals dying of caisson sickness had sufficient features in common with  $O_2$  poisoning to suggest that the increased  $O_2$  tension in compressed air may contribute to the pathology of caisson sickness.

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In the rabbits—placed in separate cages with these three dogs—the first symptom of significance was marked respiratory dilatation of the alae nasi on about the sixth day of exposure. This respiratory disturbance progressed to one of distress, a gasping inspiration with involvement of accessory muscles of respiration. Cyanosis was present in all three rabbits by the seventh day and two died. The third was taken from the chamber at this time, but had a convulsive seizure and died a few minutes after exposure to normal air. The autopsies revealed pathology very similar to that found in the dogs. Guinea pigs survived  $O_2$  for four days and the lungs at that time had the gross appearance of liver and sank in fixing fluid. Two mice survived the  $O_2$  for six days, lung pathology was similar to that of the guinea pigs. Two mice kept as controls in a similar chamber remained normal.

These investigators questioned whether their findings might not have been caused by some impurity in the  $O_2$  due to the method of its preparation (Burrows, 1917) and therefore ran a series of tests on mice. They found that  $O_2$  prepared by air reduction, by the electrolytic process, and that purified by bubbling through olive oil to remove any traces of ozone, all produced the same effects, thus ruling out the possibility that the pathology might have been caused by some impurities in the  $O_2$ . The authors agreed with the view that  $O_2$  in concentrations greater than 60 per cent of an atmosphere causes a rapid deterioration in the mammalian organism, and in concentrations over 70 per cent is poisonous to dogs, rabbits, guinea pigs and mice. The poisonous effects manifest themselves in drowsiness, anorexia, loss of weight, increasing dyspnea, cyanosis and death from oxygen want because of a destructive lesion of the lung. This lung alteration was not characteristic of an infectious process and was interpreted as a possible protective reaction which, progressing too far, causes death. "The organism, in its unsuccessful effort to achieve a new equilibrium, is kept alive by the very environmental condition which ultimately destroys it."

Faulkner and Binger (1927) found, however, that frogs were not noticeably affected by  $O_2$  in concentrations as high as 95 per cent. Turtles likewise appeared to be unaffected at their usual body temperature, but if the body temperature was raised to that of mammals, the general response to  $O_2$  and pulmonary pathology was essentially the same as that of mammals, young turtles were more resistant to this change than the older ones. In looking for an explanation of these effects the authors suggested that perhaps a reaction takes place between  $O_2$  and pulmonary tissue, the temperature coefficient of which is such that it occurs at body temperature of mammals but not at room temperature, or that a chemical irritant is produced which acts on the lung or that the metabolic rate is raised, thereby increasing the toxicity of  $O_2$ .

Achard, Binet and LeBlanc (1927), experimenting on guinea pigs and rabbits, also found that 80 per cent  $O_2$  caused distinct pulmonary pathology and death. Guinea pigs survived three to five days and rabbits about five to six days. In addition to the pulmonary congestion, desquamation and consolidation described by other investigators, the alveolar walls were found to be infiltrated with leucocytes and eosinophils. Hubbs (1930), experimenting with guinea pigs, found

dioxide, concluded that the lethal action of these substances on aquatic animals must be caused by "nascent oxygen "

The evidence which had accumulated up to this time would seem amply to warrant convicting  $O_2$  as a dangerous agent, but Smith, Heim, Thomson and Drinker (1932) were unconvinced and suspected that the evils of excess  $O_2$  had been exaggerated. Their skepticism was not unlike that voiced by Binger, Faulkner and Moore (1927) "No previous work on the toxicity of increased oxygen tension in the respired air," said Smith et al, "has been conducted under conditions in which all the other factors were constantly and perfectly controlled, and most of the investigations on this subject have been based upon data obtained from observing a limited number of animals." They decided to gather new data. The animals chosen for their investigation were albino rats from Wistar stock. These were exposed to compressed air at 3040 mm Hg pressure (an  $O_2$  partial pressure of 635 mm Hg, i.e., an  $O_2$  equivalent of 83.6 per cent at normal barometric pressure). Since the work was intended as a very careful study on  $O_2$  effects it is perhaps unfortunate that the possible complicating influence of increased  $N_2$  concentration and physical factors of increased air pressure were not ruled out by the use of  $O_2$  mixtures at atmospheric pressure.

The pathology found by Smith et al in rats exposed to compressed air and attributed by them solely to the increased partial pressure of  $O_2$ , was very similar to that observed by other investigators in animals exposed to increased concentration of  $O_2$  at atmospheric pressure. In adult rats (over 5 months old) there was dilatation of the heart, congestion of the viscera and some tubular damage in the kidneys manifested by areas of degeneration and the presence of mitotic figures in the tubular epithelium. The lungs were dark beefy red and very intensely edematous. There was pleural effusion, and considerable emphysema was present in the alveolar ducts. The capillaries of alveolar walls were engorged with R.B.Cs and the arteries and veins were surrounded by large zones of perivascular edema with varying degrees of cellular infiltration. The trachea, bronchi and bronchioles were not affected.

Young rats under forty days of age showed no evidence of this  $O_2$  damage except for an early perivascular edema, dilatation of the lymphatics, and slight cellular infiltration, in rats 100 days old these changes were more pronounced and in addition there was some desquamation, hyperplasia and hypertrophy of alveolar cells. Numerous mitotic figures were found in the alveolar walls of rats which had apparently become adapted to the increased pressure and the hyperplasia persisted for months after the rats were returned to normal air pressure. It was this increased pulmonary "cellularity," believed to be induced in adult rats by  $O_2$  at increased tension, but normally present in young rats, which the authors claimed was responsible for an increased resistance and adaptation to  $O_2$  poisoning, this adaptive change, it was said, prevented the development of  $O_2$  poisoning on re-exposure (see section on acclimatization). These authors maintained that the higher mortality in older non-adapted rats was to be explained by the loss of the young lung characteristics.

Boycott and Oakley (1932) concluded that the cause of death in rats exposed

to increased percentages of  $O_2$  at atmospheric pressure was massive pleural effusion. In  $O_2$  concentrations of 95 to 99 per cent about one-half of their experimental animals (rats) died, but no symptoms appeared in less than two or three days. On the fourth day all the animals developed progressive dyspnea, in the next two days some became worse and died while others recovered. Dyspneic rats brought into normal air died within one-half hour. The thorax was found to be full of clear liquid and the lungs completely collapsed. Capillaries were congested, fluid was found in alveoli, many monocytes but no polymorphonuclear leucocytes were found in the lung. The rest of the body (except for a general congestion attributable to circulatory failure) appeared normal. These authors suggested that the high concentration of  $O_2$  irritates the endothelium of the pulmonary capillaries so that it lets through everything in the blood plasma except the cells. Another change noted by these investigators was that white rats kept in  $O_2$  in concentrations of 65 per cent and up had a patchy yellowing of the fur.

Pflesser (1937) reported that mice breathing  $O_2$  in concentrations of 86 to 92 per cent at atmospheric pressure died in about 35 to 45 hours, cats exposed to  $O_2$  in concentrations of 93 to 97 per cent died in 70 to 86 hours. The cause of death was failure of the heart and circulation and edema of the lung with pleural exudate. Orzechowski and Holste (1938) concluded from experimental studies, that the minimum lethal  $O_2$  concentration for the rat was determined in large part by the total air pressure—an observation which points to the possible danger of making quantitative inferences concerning the effects of  $O_2$  percentage from compressed air data. Clamann and Becker Freyseng (1939) conclude from animal experiments that inhalation of  $O_2$  rich atmospheres containing more than 60 per cent  $O_2$  at atmospheric pressure leads to the death of the animal in hours or in a few days and that the cause of death is primarily lung pathology due to the action of  $O_2$  and not to any contaminations in the gas.

Armstrong (1938) states that breathing pure  $O_2$  has caused fever within six hours in both man and experimental animals. His animals developed pneumonia in from 12 to 36 hours of exposure to  $O_2$  at atmospheric pressure and died in an average of 72 to 90 hours of exposure. The pathology was essentially that as described by earlier investigators—irritation of the respiratory tract with congestion, edema, pneumonia and death. Experiments were also performed in pure  $O_2$  at decreased barometric pressures. In  $O_2$  at pressures equivalent to from 0 to 5000 feet altitudes (i.e., from 760 to 625 mm Hg) the animals (rabbits) appeared normal for 12 to 24 hours, there then occurred a slowing and deepening of respiration. Evidence of air hunger became progressively more pronounced and death occurred at zero altitude pressure in an average of 84 hours, and at 5000 feet altitude pressure in an average of 156 hours. The lungs at autopsy were liver like in appearance and consolidated, the pleural cavity contained a small amount of colorless fluid, there was edema with leucocytes present and capillary engorgement, the large blood vessels were dilated and contained much serum. At 10 000 feet altitude pressure (513 mm Hg) a few of the animals developed symptoms of air hunger on about the sixth day and died between the eighth and tenth days,

the animals exposed to from 20,000 to 30,000 feet altitude pressures showed no abnormal behavior and no pathology, at 50,000 feet altitude pressure the animals succumbed in about three minutes and these at autopsy showed death occurred from suffocation. Having made these observations, Armstrong concluded, "From this it is evident that pure oxygen should not be administered below an atmospheric pressure of 456 mm Hg (13,465 feet)." This tension of  $O_2$ , it will be noted, is just 60 per cent of one atmosphere, a concentration which has been considered for some time as the upper limit of safety in prolonged therapeutic administrations.

Binet and Bochet (1938) and Binet, Bochet and Bryskier (1939) observed that guinea pigs exposed to  $O_2$  in concentrations of 96 to 98 per cent at first tolerate the  $O_2$  without much change, but there is a tendency to lethargy and a torporous state, alternating with periods of activity, secondarily, the torpor is accentuated, anorexia is common and the animal curls up in a ball, respiration becomes dyspneic and the animal soon dies of asphyxia. The lungs show desquamation, congestion, thickened alveolar walls, leucocytic and eosinophilic infiltration, and there is generalized congestion. In continuous administrations of  $O_2$  the toxic action appears in a relatively short time, but with intermittent inhalation the survival is much prolonged. The authors conclude that continued breathing of  $O_2$  has a definite toxic action which is manifest by respiratory, biochemical, hematologic, and histologic reactions and eventually by death. They too, maintain that a concentration of 60 per cent at atmospheric pressure is the upper limit for safe administration of  $O_2$  therapeutically.

The findings of Rehbock et al (1940) emphasize further the lung pathology arising from exposure to increased concentrations of  $O_2$  (80 to 85 per cent) at atmospheric pressure. Among their findings in animals dead on the 4th and 7th days of exposure were "generalized intra-alveolar and perivascular exudate, in animals dead on the 14th and 16th days of exposure, severe suppurative bronchitis with varying degrees of bronchiectasis, large areas of bronchopneumonia characterized by a dense intra-alveolar exudate of polymorphonuclear leucocytes." They further observed, "The arterioles and smallest arteries showed no definite changes except for slight thickening of the walls of an occasional vessel or occasional foci of hyaline change. There was a condensation of new adult connective tissue around the adventitia of the large arteries. Three animals exposed for twenty-eight days and dead on the thirtieth and forty-first days, showed extensive broncho-pneumonia with abscesses, bronchiectasis, and emphysema. The arteries and arterioles showed only the slight changes seen in previous animals." Such data suggest that death from prolonged exposure to  $O_2$  may be caused not only by acute edematous changes and characteristic "pneumonia" which result in asphyxia, but possibly also by exacerbation of pathologic processes—particularly those of suppurative character—previously resident in the lung. On the other hand the experiments of Marcheaux (1943) on rats indicate that previous damage induced in lungs by vagotomy does not augment the susceptibility of those lungs to the adverse effects of  $O_2$  tension.

Bennett and Smith (1934) described progressive sclerotic changes, thickening,

narrowing and hyalinization in the pulmonary arterioles and arteries of rats, commencing as early as the third day of exposure to compressed air at 3040 mm Hg ( $O_2$  equivalent 83.6 per cent at atmospheric pressure). The changes appeared to these authors to be similar to the renal arterial lesions seen in patients dying of malignant hypertension. Dilatation of the right ventricle found in their experimental animals was attributed to the pulmonary vascular lesions. These changes were considered as having been caused by the increased  $O_2$  tension.

Rehbock, Oldt and Dixon (1940), however, found no indication of any sclerotic changes in animals exposed to  $O_2$  at from 80 to 85 per cent for periods of from 4 to 28 days. The perivascular fibrosis described by Bennett and Smith (1934) and Smith, Bennett, Heim, Thomson and Drinker (1932) in the compressed air experiments was found, but no medial or intimal changes were observed. A very definite right ventricular hypertrophy was demonstrated in animals on which autopsies were made 7 to 41 days after the beginning of the  $O_2$  exposures, a less significant right ventricular hypertrophy was present in the animals 7 to 14 months after the exposure. The examination of animals after survival periods of from 7 to 15 months likewise showed no noteworthy changes in the arteries or arterioles except for a possible increase in the collagenous tissue of the adventitia in larger vessels.

Paine, Lynn and Keys (1941) have contributed still further confirmation of the damaging effects on breathing  $O_2$  in high concentrations. In a series of experiments on 49 dogs they found that definite pulmonary changes resulted from breathing  $O_2$  in concentrations of 95 to 100 per cent at atmospheric pressure for periods as short as two hours. One dog died after only six hours of exposure and at autopsy showed the typical pulmonary pathology, but the average duration of survival in concentrations of from 95 to 100 per cent was 39 hours. These experiments demonstrate again the wide individual variation in susceptibility to the action of  $O_2$ . Oxygen concentrations of from 75 to 90 per cent produced pathologic changes similar to those seen in the 95 to 100 per cent concentrations, but the time of onset was more delayed. Occasional interruption of  $O_2$  administration delayed the onset of toxic symptoms and prolonged the period of survival. The symptomatology and pathologic findings reported by Paine et al. are in agreement with those of earlier investigators but they also included cyanosis of the muscles and abdominal viscera, gaseous distention of the stomach and in some cases there was a marked atrophy of the hepatic parenchyma in the central part of the lobules said to be comparable to that seen in right heart failure.

Definite pathologic lesions were found even in exposed animals which had shown no symptoms of the damaging action of  $O_2$ —an observation of interest in relation to the use of high concentrations of  $O_2$  in therapy. The authors say, "It is not impossible that most or all of the pathologic changes, which we have observed, might be explained by an hypothesis of deranged vascular physiology." While the number of animals used in this investigation contribute in large measure to the value of the experimental results, it is perhaps to be regretted that no mention has been made of controls which Karsner (1916) found were so very important in pathologic studies of  $O_2$  poisoning in laboratory animals. The



CO<sub>2</sub> content of the respired air in these experiments varied between 0.2 and 2.0 per cent of an atmosphere

Kaunitz (1942) reported that subjecting mice to pure O<sub>2</sub> at atmospheric pressure for two or three days results in myocardial lesions consisting of "marked degeneration and fragmentation of the muscle fibres, some of which show granulation and loss of striation. Capillary dilatation presumably due to congestive heart failure is also to be noted." It was maintained that while at first the O<sub>2</sub> reaches the alveoli with ease, the bronchi later go into spasm, become filled with mucus and so obstruct the air passages that the resulting decrease in intra-alveolar pressure causes the alveoli to collapse, the experimental evidence of this sequence of events is not presented. The myocardial lesions were said to be caused by the anoxemia consequent upon the pulmonary lesions. The number of animals examined and the condition of the controls, if any, were not stated. Pichotka (1941) and Liebegott (1941) also reported cardiac pathology including leucocytic and lymphocytic infiltration, and necrosis of muscle fibers, and attributed this to hypoxemia arising from the lung damage induced by the O<sub>2</sub>. Rehbock et al (1940) found ventricular hypertrophy in dogs exposed to 80 to 85 per cent O<sub>2</sub>.

Marechaux (1943) described pathological changes in rat lungs as a result of exposure to O<sub>2</sub> in concentrations of 81.5 per cent, in addition to the more usual changes previously described there was edema of the blood vessel walls (gefäßwandödem) and border striations (randstreifen) in the alveolar walls which the author suggested were comparable to the "Quellungs Nekrosen der Alveolarwände" previously described by Liebegott (1941), Clamann, Becker-Freyseng and Liebegott (1942). Pichotka (1941) had also noted this "Quellungs Nekrosen" in addition to other pathology and pointed out that the lung picture was similar to that of phosgene poisoning, pneumococci type X were also observed. Behnke and Willmon (1941) describe what they believe to be a new clinical entity of delayed pain, congestion and hemorrhage in the middle ear following O<sub>2</sub> inhalation during high altitude pressure changes, these effects which develop chiefly during sleep have been attributed by the authors to a reduction in the middle ear pressure resulting from the absorption of the contained O<sub>2</sub>.

In summary then the more outstanding pathology as revealed by tissue examination would include the following: inflammation, congestion, edema, atelectasis, fibrin formation and consolidation in the lungs, pneumonia of various types, bronchitis with bronchiectasis, hypertrophy, hyperplasia, desquamation and degenerative changes in alveolar cells, sclerotic changes with narrowing, thickening and hyalinization of pulmonary arterioles, dilatation of the right or both sides of the heart, cardiac hypertrophy and cloudy swelling, congestion of abdominal viscera with cloudy swelling in the kidneys, splenic contraction and testicular degeneration. The long accumulation of experimental data leaves little question but that continuous exposure to O<sub>2</sub> in concentrations above 60 to 70 per cent at atmospheric pressure for from 12 to 14 hours or even less, results in pathological changes particularly in the lung, and that if exposure is further prolonged these changes frequently prove fatal. The onset of the noxious effect on

man may be more delayed than in other animals, but there is good reason to believe that the human animal is similarly affected (See section on noxious effects of  $O_2$  at atmospheric pressure and  $O_2$  therapy)

*Acclimatization and Tolerance* Campbell (1927) observed a decrease in breathing of as much as 30 per cent in animals exposed to an  $O_2$  rich atmosphere. It was claimed that this change in ventilation together with an observed decrease in both the hemoglobin per cent and R.B.C. count, constituted a mechanism by which the organism attempted to acclimatize itself to the high  $O_2$  tensions. It was further suggested that vascular changes and a shift in the dissociation curve of hemoglobin might also contribute to this acclimatization whereby an excess uptake of  $O_2$  by the tissues might be prevented. These mechanisms, however, quite evidently did not succeed in holding the  $O_2$  level of the tissues at their normal values, for both the  $O_2$  and the  $CO_2$  tensions of the tissues were found to be increased as a result of breathing the hyperoxygenated air. When the animals were returned to normal air there was hyperpnea and a distinct drop in the tissue  $CO_2$  tension—a reaction which was interpreted to mean that now the  $O_2$  tension of normal air was insufficient normally to sustain the  $O_2$  acclimatized animal. In any case this post-exposure hyperpnea indicates that during the exposure to the hyperoxygenated air there had been some adjustment to the  $O_2$  enriched air which was incompletely or slowly reversible, and the fact that the  $O_2$  concentration employed was distinctly less than that which it is generally agreed causes lung changes, would localize this acclimatization to some tissue beyond the lung membranes. Campbell suggests that the hemoglobin forming organs are regulated by the  $O_2$  tension, but Karsner's (1916) examination of blood and tissues, including that of bone marrow, revealed no evidence in support of that suggestion.

In their entirety, the reports on red cell and hemoglobin content of the blood hardly justify a conclusion that there is any one predominant adaptive response in the hematopoietic system to breathing  $O_2$  or hyperoxygenated air, on the other hand the consistent finding of a decrease in red cell size and a common, though not invariable, leucocytosis, is highly suggestive of the operation of some more or less universal adaptive mechanisms which affect the cellular characteristics of blood.

Smith (1899) suggested that the pulmonary pathology induced by exposure to  $O_2$  might act as a protective barrier against the entrance of too much  $O_2$  into the blood. Such changes have been referred to by some authors as an "acclimatization,"—perhaps a misuse of the term, since those changes commonly prove fatal during the animal's sojourn in  $O_2$  or after its return to normal air.

Barach (1926) in an attempt to determine whether the lungs might be capable of acquiring some degree of acclimatization or immunity to the injurious effects of  $O_2$  in high concentrations, subjected rabbits to a gradually increasing  $O_2$  concentration from 21 per cent to 85 per cent, or to a maintained high  $O_2$  percentage just below toxic concentrations. No evidence of acclimatization was obtained, subsequent exposure to  $O_2$  in concentrations of 80 to 85 per cent invariably produced a serous pneumonia.

Boycott and Oakley (1932) observed that if rats, dyspneic from an exposure to

O<sub>2</sub> at increased concentrations were brought into ordinary air, many of them died within half an hour, "Their pulmonary condition is evidently such that they can survive only in high concentrations of oxygen" The survivors of O<sub>2</sub> exposure experiments continued to live normally in 95 per cent O<sub>2</sub> for some time though they were still liable to die on coming into air, but after about a month or six weeks they began to deteriorate "They become pecky, deteriorate in morale and do not clean themselves, lose their appetites, waste and die, and in this sequence of events there is much individual variation They do not become anaemic and at post mortem there is no obvious cause of death, though in one or two instances there has been a small pleural effusion" Controls living for longer times under exactly the same conditions in 50 to 55 per cent O<sub>2</sub> remained well The authors refer to this post-exposure deterioration as "chronic O<sub>2</sub> poisoning"

That changes take place in an animal as a result of exposure to O<sub>2</sub> enriched air which are not readily reversible when returned to normal air is shown again in the experiments of Binger, Faulkner and Moore (1927), a rabbit exposed to 70 per cent O<sub>2</sub> at atmospheric pressure for three days had a convulsive seizure and died when it was taken from the O<sub>2</sub> chamber and replaced in room air

Smith, Heim, Thomson and Drinker (1932) and Smith, Bennett, Heim, Thomson and Drinker (1932) maintain that acclimatization or adaptation takes place in the lungs of rats on prolonged exposures to increased O<sub>2</sub> concentrations By the fourth day of exposure to compressed air at 3040 mm Hg (an O<sub>2</sub> equivalent of 83.6 per cent of one atmosphere) they found pronounced symptoms of O<sub>2</sub> poisoning, anorexia, loss of weight, respiratory embarrassment, hyperpnea and cyanosis, in rats over three months of age, 13 per cent succumbed The surviving rats showed improvement by the sixth day although there was still anorexia and weight loss, but the animals of this group sacrificed for autopsy, showed that the pathological changes found in the third and fourth day rats, were missing Young rats (under three months of age) showed a depression in normal growth rate, but appeared to be unaffected otherwise

After 72 days of exposure, decompression to atmospheric pressure was carried out, 28 per cent of those still alive at the end of the 72 day exposure died during, or within twenty-four hours after, their return to normal air pressure and at autopsy all of these showed severe lung damage, particularly broncho-pneumonia The survivors of decompression showed no signs of O<sub>2</sub> poisoning when, after a 40 day interval in normal air, they were re-exposed to compressed air, and only 2 in a litter of 8 born during the second exposure died, whereas during the first exposure all of 9 litters born, died The absence of any fatalities on decompression from this second exposure to compressed air is in contrast with the high mortality (28 per cent) on decompression from the first exposure It may be noteworthy, however, that the first exposure was seven times longer than the second The authors interpreted their results as evidence that during the first exposure there had developed an increased resistance to the toxic effects of O<sub>2</sub> which was of the nature of a permanent adaptive change

The essential feature of this adaptive reaction was described as a pulmonary change, characterized by hypertrophic and hyperplastic alterations in the pul-

monic capillary bed and in the properties of the alveolar cells. The thickening and increased "cellularity" of the alveolar walls was similar to that found in normal non-exposed rats under 3 months of age. It was thought that this peculiar morphology, considered as having been developed in the adult rats by  $O_2$  exposure, but normally present in young rats, was the cause of the greater  $O_2$  resistance observed in the adult adapted, and in the young unadapted rats. If this interpretation be correct, one may perhaps with reason ask why all of nine litters born during the first exposure to compressed air succumbed, unless, of course, the peculiarly resistant morphology is absent in very young lungs.

This evidence presented by Smith et al (1932) does unquestionably suggest there is some pulmonary adaptation to increased concentrations of  $O_2$ . But the high mortality met with in the process of its development (13 per cent early in exposure and an additional 28 per cent on return to normal air) indicates that this adaptive response is either absent in many individuals or that there is a very wide variation in its effectiveness, some of the data may perhaps be also interpreted as a reflection of a selective process. The recognized wide individual variation in susceptibility to  $O_2$  poisoning, the pathological findings in so-called normal animals (Karsner, 1916) and the fact that practically all those rats which succumbed showed broncho-pneumonia, might well imply that a selective process was operative and that possibly the toxic action of  $O_2$ , superimposed upon a pre-existing sub-symptomatic pathology was the cause of death. Since however these experiments were carried out in compressed air, rather than in  $O_2$  or hyperoxygenated air at atmospheric pressure, some degree of reservation may be called for in assuming that the results are attributable solely to the increased  $O_2$  tension.

Nelson and Gowen (1930) reported that the incidence of "spontaneous" pneumonia in rats increases with age up to one year, at which time about 75 per cent of the animals are affected, in young animals, less than three or four months old, the occurrence of pneumonia was relatively infrequent. This again suggests the possibility that the greater resistance to  $O_2$  poisoning observed by Smith et al in rats under three months of age might have been due to an absence of pre-exposure lung pathology in their young animals. The surprisingly extensive involvement of the lungs necessary to induce any symptoms of pulmonary pathology in rats (Griffith and Farris, 1942) emphasizes the impressively wide margin of safety provided in the lungs and the danger, where autopsies are not performed, of assuming that an absence of signs or symptoms is indicative of either an immunity to the effects of increased  $O_2$  tension or an absence of pulmonary damage induced by  $O_2$ .

Rats advanced in pregnancy were reported (Smith et al, 1932) to be more susceptible to  $O_2$  poisoning in compressed air than non pregnant, owing, it was thought, to encroachment upon the thoracic cavity by the gravid uterus, but Ozorio de Almeida (1934) found that pregnant rats were no more susceptible to the toxic effects of  $O_2$  at high barometric pressure than the non pregnant.

Evidence has already been cited that exposure to  $O_2$  at increased tension depresses metabolism. Such a reaction in itself might conceivably be interpreted as a protective or adaptive response and suggests that an organism's basal

metabolic rate may be intimately concerned with its susceptibility to the adverse effects of  $O_2$  at increased tensions. Evidence that this is so is found in several reports. Faulkner and Binger (1927) observed that raising the temperature of turtles increased their susceptibility to the adverse effects of prolonged breathing of  $O_2$  enriched air at atmospheric pressure, and Campbell (1937) noted a similar effect of increased temperature in mammals.

Clamann and Becker-Freyseng (1939) maintain there is an inverse relationship between the size of an animal and its susceptibility to the deleterious action of  $O_2$ , and this, it is claimed, is related to the lower metabolism of large animals in terms of their body surface. Binet, Bochet and Bryskier (1939), however, found that in  $O_2$  concentrations of 90 per cent at atmospheric pressure, mice survived longer (140–150 hrs) than guinea pigs (60–70 hrs). Ham and Hill (1906) and Hill (1912c) likewise reported that small animals are not more susceptible than large. Thompson (1889) found the monkey was affected before the pigeon, and the dog before the guinea pig. Smith (1899) found no relationship between the size of the animal and its susceptibility to  $O_2$  poisoning. Nor does the data of Soule (1939) concerning the species' susceptibility show any distinct relationship between size and survival in  $O_2$  exposures, mice survived as long as rats.

It has been widely accepted that basal metabolism decreases with the approach of maturity, in man and other animals (Magnus-Levy and E. Falk, 1899, Aub and DuBois, 1917, Kise and Ochi, 1934, Matson and Hitchcock, 1934, Lewis, 1938, Davis and Hastings, 1934, Benedict and Macleod, 1929, Kibler and Brody, 1942, and others). If then there is a direct parallelism between the susceptibility of an animal to the adverse effects of  $O_2$  and its basal metabolism, young immature animals should be more affected by  $O_2$  than older full grown animals but this is contrary to the findings of Smith et al (1932) in rats and those of Thompson (1889) and Binet, Bochet and Bryskier (1939) in pigeons. Paine et al (1941) observed however that young pups were just as susceptible as full grown dogs. The factors of growth and immaturity no doubt introduce some peculiar influence which further complicates the relationship between body size, metabolism and susceptibility to the toxic action of  $O_2$ .

Interestingly enough Benedict and Macleod (1929) claimed that the generally accepted interpretation that the metabolism of smaller animals is greater than that of large animals is erroneous, they found that in terms of surface area the metabolism of the white rat is actually less than that of man. But Dubois (1929) maintained that little animals have a higher metabolism and respiration per unit weight than large animals so that their tissues should become more rapidly saturated with the respired gas. Since there is some disagreement among authorities concerning the relationship between body size, inherent metabolism and the best method of expressing that relationship, especially where different classes and species are concerned (Krogh, 1916, Benedict and Macleod, 1929) it would appear wise to exercise caution in the interpretation of differences in susceptibility to  $O_2$ , on the basis of reported differences in basal metabolism. If small animals are more susceptible than large, perhaps a greater rapidity of blood circulation is causally involved in addition to possible differences in metabolism.

Soulie (1939) reported that when animals are exposed in alternating periods of 24 hours' duration to normal air and to  $O_2$  in high concentrations (90 to 100 per cent at atmospheric pressure) there occur no indications of any physiologic disturbance or  $O_2$  poisoning, but if after about six weeks of such treatment the animals are continuously exposed to the increased  $O_2$  concentration they react and die just as untreated animals do. If, however, continuous inhalation of high concentrations of  $O_2$  at atmospheric pressure be carried to the point of appearance of pulmonary symptoms and this is repeated intermittently, the resistance of the rat to  $O_2$  poisoning can be augmented. It was also maintained that a similar augmentation of resistance to increased  $O_2$  per cent can be accomplished by inhalations of sublethal concentrations of disphogene but guinea pigs do not develop tolerance by these methods as well as rabbits.

The report of Rehbock, Oldt and Dixon (1940) is of interest in relation to the question of adaptation. These investigators found that rats, dead on the fourth day of exposure to  $O_2$  in concentrations of 80 to 85 per cent at atmospheric pressure, showed the characteristic pulmonary pathology ("fibrinous pneumonia") arising from such treatment, those animals dead on the seventh day of exposure showed the same but less severe damage. In animals dead on or after the fourteenth day of exposure these changes were absent although there was other pathology present. The diminishing severity of the "fibrinous pneumonia" with prolongation of exposure may be variously interpreted, it may represent a progressive development of pulmonary resistance or acclimatization to the toxic action of  $O_2$ , or again it may indicate simply a selective action of the toxicity for the more susceptible individuals.

The best explanation for those respiratory difficulties so frequently experienced by animals on their return to normal air after a prolonged exposure to  $O_2$  or hyperoxygenated air at atmospheric pressure and which with some stretch in the meaning of the term might be considered a manifestation of "acclimatization," would appear to lie in the pulmonary pathology which such exposure is known to produce. Certainly the changes in hemoglobin and red cell content of the blood mentioned above could hardly account for the post exposure respiratory difficulties. There is, however, another possible contributor less obvious than that of lung damage which may deserve some consideration, namely, an alteration in fundamental enzymatic processes in the tissues. The impressive evidence that increased tensions of  $O_2$  inhibit and even irreversibly damage some of the respiratory enzymes provides reasonable basis for the speculation that such enzymatic changes may contribute not only to acclimatization, but also to the post-exposure reactions. The report of Boycott and Oakley (1932) cited above that animals deteriorated and died of "chronic  $O_2$  poisoning" without showing at autopsy any obvious cause of death is of interest in this connection.

The reports that patients to whom  $O_2$  or hyperoxygenated air has been administered therapeutically, occasionally have difficulty in readjusting to ordinary air, constitute further argument that some adaptive changes of slow reversibility, similar to that occurring in experimental animals, and quite aside from pulmonary alterations, are evoked by the  $O_2$  treatment. Armstrong (1939) observed that

animals which had shown symptoms of pneumonia as a result of exposure to pure  $O_2$  at normal or reduced barometric pressures died almost immediately when removed to normal air. This was interpreted as due to an alteration in the diffusion of  $O_2$  consequent upon the pulmonary inflammation induced by the  $O_2$  and led Armstrong to suggest that pneumonia patients receiving  $O_2$  therapeutically should never have the  $O_2$  supply stopped suddenly.

Additional evidence that the return to breathing normal air after exposure to  $O_2$  at one atmosphere causes some disturbance is found in the report of Behnke et al., (1934) that while a subject felt well during the inhalation of  $O_2$  he experienced substernal discomfort and coughed during the first half hour after his return to normal air. It has also been occasionally observed that after long periods of  $O_2$  administration in anesthesia, a sudden removal of the mask is followed by profound circulatory disturbances, particularly an extreme drop in blood pressure. These effects imply a lag in reversibility of adaptive changes other than pulmonary. Waters (1942) questions whether such disturbance might not represent the inability of the organism to readjust immediately to the normal biochemistry of blood and tissues which have become accustomed to high tensions of  $O_2$  and  $CO_2$ .

**NOXIOUS EFFECTS OF  $O_2$  AND THERAPY** Some of the early advocates of the use of  $O_2$  for therapeutic purposes (Priestley, 1775, Beddoes and Watt, 1796, Beddoes, 1797) recognized that breathing pure  $O_2$  might cause lung damage. This recognition exerted a damping influence on the therapeutic use of  $O_2$  which, since Priestley's announcement of its possible salubrious action, had been carried to extremes not only by charlatans but also by physicians of better repute.

The first flush of enthusiasm for  $O_2$  therapy had about died down by the end of the first decade of the 19th century, but there was a distinct resurgence some forty years later, so that shortly after the middle of the 19th century,  $O_2$  therapy—or its handmaiden, compressed air therapy—had been reported as successful, sometimes fantastically so, in the treatment of practically every one of the maladies to which human flesh had fallen heir. This revival of  $O_2$  therapy was particularly evident in England (Birch, 1859), and in France, Demarquay (1866) expressed astonishment that in spite of its beneficial effects described by Beddoes (1797) and others,  $O_2$  "should have been abandoned as a curative means" and treated as a dangerous gas because of its activity in chemical combustion.

The value of  $O_2$  as a means of combating hypo-oxemia was early recognized and its use in the treatment of pneumonia advocated (Beddoes, 1797, Golden 1866, Brunton and Prickett, 1892, Brunton, 1912, Hill, 1912 and others). Cabell, (1874) described several cases in which the administration of pure  $O_2$  had been a life-saving measure and recommended its use even though he stated "In general the use of the gas is held to be contra-indicated in the presence of acute inflammation." He agreed with Smith (1870b) who maintained that "When respiration is seriously interfered with, the danger from this source outweighs all risk from any possible increase of the inflammation which the use of oxygen may occasion." The method of  $O_2$  administration at that time however was not one of continuous exposure for prolonged periods so that chances of injury from  $O_2$  were very remote as were also, in many cases, possibility of benefit to be derived

Fauntleroy (1916) reported that  $O_2$  treatment in injuries from chlorine and bromine gas was distinctly beneficial. Haldane (1927) recommending  $O_2$  for the relief of anoxemia arising from gas poisoning in World War I, cautioned that in some cases it might cause further damage in a lung already inflamed.

Stadie (1919, 1922) emphasized the relationship between the degree of hypoxemia and the mortality in pneumonia. His construction of an  $O_2$  chamber for the treatment of this condition by  $O_2$  in concentrations up to 65 per cent at atmospheric pressure represents one of the more careful and consistent studies made in this country on the application of  $O_2$  in therapy. The consensus of opinion then and in the years following was that continuous exposure of an individual to  $O_2$  in concentrations much above 60 per cent at atmospheric pressure may lead to adverse and deleterious effects on the lungs.

In support of that opinion there has since accumulated the considerable volume of evidence from animal experimentation mentioned above. Barach (1926, 1934) from his own experimental animal studies and from practical clinical experience has maintained over a period of years that "Very rich oxygen mixtures, such as those between 80 and 100 per cent, have repeatedly been shown to produce irritant inflammatory lesions in the lungs when used continuously for two or three days or more, but concentrations under 70 per cent have no such influence. Furthermore, there is no demonstrable *tendency* to produce edematous changes in the lungs when atmospheres containing less than 70 per cent oxygen are employed. The use of oxygen concentrations between 90 and 100 per cent in human beings for long continuous periods is entirely unwarranted by the present experimental data." DuBois (1929) maintained the optimum effect for  $O_2$  administration is 40 per cent. Richards and Barach (1934) found that living for one week in an atmosphere containing 45 per cent  $O_2$  caused no indication of irritative action on the lungs of two normal young men.

Boothby (1932) likewise has long cautioned against the use of  $O_2$  in the higher concentrations and states that  $O_2$  "in excess of 450 mm. corresponding to 60 per cent at sea level should be rigorously avoided."

The fact that some patients who have died of pneumonia in spite of  $O_2$  administration have shown more extensive lung damage than those dying without  $O_2$  treatment, might seem to indicate that such treatment was the cause of the extension, but Boothby (1932) accepts Robertson's (1932) suggestion that such results may also mean that the  $O_2$  treatment prolonged the life of the patient so that the pneumonia was able to progress to more advanced stages. Moersch (1937) recommends the use of  $O_2$  in concentrations of from 30 to 60 per cent and maintains that concentrations above this offer no additional value and actually may be harmful if used for any length of time, due to the irritative action on the lungs.

Evans (1927) argued that there was no proof that a normal individual is harmed by breathing  $O_2$  in concentrations as high as 80 to 100 per cent for one or several days. He also maintained that the data from research done on normal animals are not applicable to animals or men suffering from diseased conditions of the lungs. The work of Karsner (1916) however emphasized the fact that a large percentage of so-called "normal" experimental animals are actually suffering from



sub-symptomatic lung pathology, this would certainly indicate that the adverse effects of  $O_2$  are by no means limited to normal lungs and that animals with pulmonary lesions, as well as normal animals are adversely affected by, and may die as a result of,  $O_2$  exposure

It was further reported (Evans and Durshordwe, 1935) that  $O_2$  in concentrations of from 70 to 100 per cent had nothing but beneficial effects on the lungs of pneumonia patients and that  $O_2$  at 100 per cent administered for from one to four hours daily not only was without harmful effects but that it induced excellent therapeutic results. Considering the numerous investigations which had previously demonstrated the relief of hypo-oxemia and the beneficial effects in pneumonia induced by  $O_2$  administration, such results were only as might be expected.

In view of the reasonableness of the use of  $O_2$  at high concentrations in many emergencies it is perhaps unfortunate that, to support his belief in the therapeutic use of 100 per cent  $O_2$ , Evans (1939) has maintained that those opposed to an indiscriminate use of 100 per cent  $O_2$  do so on the basis of what he calls the "healthy animal theory." Perhaps the seemingly misleading implications of Evans' argument may be sufficient excuse for its presentation here. "The healthy animal theory," says Evans, "assumes that facts concerning human beings and the lower animals are interchangeable and that data obtained by experimentation upon animals would, therefore, apply to humans. This assumption is contrary to the facts, as there has never been any uniformity in the reaction of humans and the lower animals to drugs or other therapeutic agents. The second part of the theory assumes that if a given dosage of oxygen is harmful to a healthy animal, it would also be harmful to an anoxemic patient. Take, for example, the dosage of insulin required for the successful treatment of diabetic coma. The dosage in these cases far exceeds the safe dosage for normal individuals. The third part of the theory assumes that the tolerance of healthy animals to oxygen represents dosage for the treatment of anoxemia." The argument is obviously spurious and the analogy of insulin dosage ill-chosen.

The oxygen demands of an individual can be estimated with fair accuracy, and the  $O_2$  needed to relieve a given  $O_2$  unsaturation in pneumonia and similar hypo-oxemic conditions may be readily determined. The dilemma in the  $O_2$  therapy of such cases, then, is not the difficulty of determining the "dosage" of  $O_2$  needed but rather, how can the "dosage" be gotten across grossly defective pulmonary membranes and this without at the same time inducing injury.

A significant point which Evans seems to have overlooked in his insulin analogy is that insulin—fortunately enough—is not given by pouring it into the lungs, if it were, the problem of insulin administration would be similar to that in  $O_2$  administration and the question would be—How can the desired amount of insulin be gotten to the tissues without injuring the pulmonary tissue or drowning the patient? The voluminous literature on  $O_2$  therapy contains many reports of attempts to administer  $O_2$  by various routes.  $O_2$  baths, colonic, vaginal, subcutaneous, intramuscular, intravascular and intra-abdominal injections, the imbibition and enemata of highly oxygenated solutions, have all been tried—most of them more than a century ago—with the idea of by-passing the respiratory epithelium. As yet none of these attempts has been eminently successful.

Evans (1939) reported that in 1925 he had inhaled pure  $O_2$  for four hours a day for several days without any apparent harmful effects "This was done," he says, "In order to find a safe dosage for the treatment of disease not complicated by anoxemia." He has thereby demonstrated, notwithstanding his argument to the contrary, that he not only subscribes to, but has made practical use of, the "healthy animal theory." His claims, however, that the pneumonic lung has a higher tolerance for  $O_2$  than a normal lung because of the fever temperature and the attendant increase in metabolism, is contrary to the observation of Faulkner and Binger (1927) and of Campbell (1937a,b) that increase in temperature increases susceptibility to  $O_2$  poisoning.

The relative harmlessness of breathing  $O_2$  in high concentrations for short periods would seem to be adequately demonstrated by the work of Benedict and Higgins (1911) and by the almost universal employment of pure  $O_2$  in routine basal metabolism measurements. Hill and Macleod (1912) and Adams (1912) reported that men suffer no ill effects of breathing  $O_2$  in concentrations of 80 to 90 per cent for periods as long as two hours. Fine, Hermanson and Frehling (1938), applying Bert's principle of washing out the tissue nitrogen by breathing pure  $O_2$  to the removal of air and gaseous accumulations in body cavities, found no particularly adverse effects from breathing  $O_2$  in concentrations of 95 per cent. Similarly Schwab et al (1936) observed no pulmonary complications in encephalographic patients who had been administered  $O_2$  in similar concentrations for periods of 3 hours to facilitate the removal of entrapped air. Boland (1940) also reported that no pulmonary trouble was noted in interrupted administrations of pure  $O_2$  to patients. The application of these principles to remove tissue nitrogen in attempts to prevent aeroembolism in aviation has also been widely accepted as harmless. Behnke and Willmon (1941) found no adverse symptoms in man after breathing  $O_2$  in concentrations of 99 per cent at atmospheric pressure for 17 hours.

In recent years the urgency of the use of  $O_2$  in altitude work has led to a re-evaluation of the effects of administering  $O_2$  in very high concentrations to man. Barach and Richards (1935), in contrast with the earlier firm conviction that the use of  $O_2$  in concentrations above 60 per cent (Barach 1926, 1934) was dangerous, have modified the former view somewhat and now think that the use of  $O_2$  in 100 per cent concentrations may be safe and desirable for short periods but that "the danger of oxygen poisoning resulting in pulmonary edema, which regularly occurs in animals exposed to concentrations of oxygen over 80 per cent for two to four days, should be a sufficient warning to prohibit the employment of 100 per cent oxygen to human beings for similar periods." Barach and Eckman (1941) point out that while the literature contains frequent reference to the use of 100 per cent  $O_2$ , it is not breathed as such in actual practice because of the dilution by small amounts of  $CO_2$ , rare gases,  $N_2$ , and water vapor. They agree that  $O_2$  in percentages of from 80 to 100 per cent may be safely administered for periods of from 16 to 24 hours, but think it wise subsequently to lower the concentration below 70 per cent.

Boothby has likewise discarded his former interpretation that the use of  $O_2$  in concentrations above 60 per cent should be "rigorously avoided" and recently

stated (Boothby, Mayow and Lovelace, 1939) that 100 per cent  $O_2$  administered for 48 hours causes no signs of pulmonary irritation. These authors advise its use in a great variety of clinical conditions "traumatic shock, abdominal distention, headache following encephalography, certain types of migraine, profuse pulmonary oedema, massive collapse of the lung, pulmonary embolism, angina pectoris, and some other cardiac conditions, infections due to anaerobic organisms such as those of gas gangrene and tetanus and possibly certain infections due to partially anaerobic organisms."

Armstrong (1938), having formerly cautioned against the use of  $O_2$  at tensions greater than 456 mm Hg, more recently reports (Armstrong, 1939) that the danger of the use of high  $O_2$  concentrations has been overemphasized and says, "recent clinical experience indicates that the human lung is more resistant [than animal lungs] to the irritative effect of pure  $O_2$  and in a few cases it has been administered continuously for as long as five consecutive days without any apparent harmful effect." The drying and chapping of the lips, mouth and throat with occasional irritation extending down as far as the larynx and the hoarseness which has occurred not uncommonly in those using the plain  $O_2$  tube or the open  $O_2$  mask have been attributed to the dryness of the gas rather than to any effect of  $O_2$  itself (Armstrong, 1939). This explanation is reminiscent of the charge made by Brüning (1912) that the  $O_2$  effects reported by Bornstein and Strouk (1912) were caused by the dryness of the gas rather than the  $O_2$ —a charge which Bornstein (1912) later refuted.

The present tendency to dismiss the possibility that  $O_2$  in high concentrations exerts no significantly deleterious action on man, calls for a consideration of some of the indications that the human organism is not immune to the adverse effects of  $O_2$  in concentrations approximating 80 or 100 per cent at atmospheric pressure. Such evidence may be found *a*, in data from experimental exposures of men to pure  $O_2$  or to hyperoxygenated air, at atmospheric pressure, *b*, in occasional reports of peculiar responses in patients or normal individuals to the shift from an atmosphere of air or low  $O_2$  to one of pure  $O_2$  or hyperoxygenated air, *c*, in reports concerning the effects of increased  $O_2$  tensions in exposures to compressed air, although, as already stated, some degree of reservation is demanded in accepting such effects as those of  $O_2$  alone.

*a Adverse Effects of  $O_2$  or Hyperoxygenated Air, at Atmospheric Pressure on Man.* Conclusive evidence that men may be adversely affected by breathing  $O_2$  in increased concentrations at atmospheric pressure is presented in the reports of Behnke, Johnson, Poppen and Motley (1935) and of Behnke (1940). These investigators found that one of ten men breathing  $O_2$  at from 96 to 99 per cent at atmospheric pressure became fatigued and had symptoms of bronchial irritation after 4 hours exposure, in another series one subject complained of substernal pain and toxic effects at the end of 6 hours' exposure. Behnke and Willmon (1941) found that tests in which 99 per cent  $O_2$  at atmospheric pressure was employed, frequently had to be terminated at the end of 6 hours on the subject's complaint of substernal soreness, aggravated by deep inspiration, although auscultation and x-ray examination failed to reveal any alterations. The

authors maintained that the substernal distress points to the irritant property of pure oxygen and indicates pulmonary congestion and cardiac vasomotor spasm. It was further reported by these authors that impaired neuromuscular coordination and the power of attention, or an increased effort to maintain these functions, occurred after 1 to 3 hours of exposure in 3 of 4 subjects tested, hyperpnea and symptoms of lung irritation, dry cough, substernal pain and a high leucocyte count were also observed. The authors concluded, however, that healthy men between the ages of 22 and 40 can breathe pure  $O_2$  at one atmosphere with comparative safety for 4 hours.

Anthony (1940a) maintains that breathing  $O_2$  causes no noticeable disturbance in healthy persons, but that careful studies show that an increase in partial pressure of  $O_2$  above normal elicits responses in the human organism as consistently as does a decrease. Prolonged administration of  $O_2$ , especially if there is an associated increase in air pressure, may cause severe or even fatal changes.

Perhaps one of the most valuable and informative contributions concerning the effects of increased percentages of  $O_2$  at atmospheric pressure on the normal human lung is that of Becker Freyseng and Clamann (1939). These investigators exposed themselves to  $O_2$  (90 per cent) at atmospheric pressure for a period of 65 hours. On the second day of exposure the pulse frequency and vital capacity which had decreased initially were distinctly increased. There was also an elevation in body temperature. Paresthesias appeared in both individuals, particularly in the finger tips, spreading from the middle finger, and on the following day involved the toes. One of the subjects (B) became ill and his vital capacity then diminished progressively, pulse rate and temperature continued to rise. Pain was felt in the knees and elbows. Dyspnea became apparent but auscultation revealed nothing abnormal. The objective and subjective effects progressively increased in both individuals. The electrocardiogram showed no abnormality. On removal from the chamber the x ray revealed no lung changes in either subject. The blood examination showed an increased R B C count but the hemoglobin content was about normal. The leucocytes had increased—particularly in subject B in whom it had risen from 6200 to 12,700. Subject C felt well but the paresthesia persisted in both men for ten days. Subject B's illness which had begun in the chamber became worse on removal, he vomited, complained of nausea and headache. The following evening he was taken to the hospital, the vomiting stopped the first night, but there was a distinct bronchitis with fever. There was fluid in the right thorax and signs of bronchopneumonia but the x rays were still negative. Three days later the fever fell and he was discharged from the hospital on the eighth day. The vital capacity continued low until the sixth week. The paresthesia described by Becker Freyseng and Clamann suggests that the numbness of the lips observed by Hill and Greenwood (1906) during their exposure to compressed air and described by them as a "false sense of anesthesia" may very well have been an effect of the increased  $O_2$  tension.

The numerous reports of atelectasis among the autopsy findings of experimental animals exposed to  $O_2$  finds a counterpart in the report of Waters (1942) that in the human lung, inactive portions more readily became atelectatic when filled

with pure  $O_2$  than when they are filled with air. The possible occurrence of atelectasis may constitute a hazard in the therapeutic use of pure  $O_2$  where for any reason a portion of the lung is not freely ventilated or where the  $O_2$  may be partially or completely trapped in, and subsequently absorbed from, the alveoli of that portion (see p 147)

b *The Response to a Shift from Air or Low  $O_2$ , to Pure  $O_2$  or Hyperoxygenated Air* The literature contains several reports that the shift to hyperoxygenated air following states of hypo-oxemia or low  $O_2$  administration has resulted in peculiar and untoward reactions which are difficult to interpret except as manifestations of an inability to adjust rapidly to the higher  $O_2$  tensions, or to some adverse action of  $O_2$  itself

Barach (1941) has reported that patients who had suffered for some time from anoxic conditions frequently showed profound mental disturbances lasting several days when subjected to  $O_2$  in concentrations of about 50 per cent at atmospheric pressure. One such patient whose arterial  $O_2$  saturation was only 55 per cent and to whom 50 per cent  $O_2$  was administered reached a state of complete irrationality and delirium within one hour of the initiation of the treatment. Dill (1941) discussing these observations points out, however, that residents of high altitudes are not apparently affected adversely by their return to normal atmospheres after having been acclimatized to low  $O_2$  atmospheres. This suggests that perhaps the rate of change in gaseous composition of the air breathed may be involved.

Schwarz and Mahkiosis (1938) in altitude tolerance tests found that the sudden elevation of  $O_2$  tension after periods of hypo-oxemia resulted in deterioration and disorders of the subjects. Moody and Howard (1942) reported that  $O_2$  administered by tent to a 2 year old child suffering pneumonia caused convulsive attacks. The first exposure was continuous for 48 hours, at the end of which time the patient was removed to room air, then returned to the  $O_2$  at intervals for 6 days. There was original relief from the pneumonia but in one of the exposures the patient developed severe convulsions which involved the whole body, the temperature rose to  $101^\circ F$ . Removal from the tent resulted in cessation of the attack but dyspnea again developed necessitating the patient's return to the tent in the afternoon, this precipitated another convulsion which stopped almost immediately on being removed from the tent. The  $O_2$  concentration was in the neighborhood of 72 per cent.

These observations are reminiscent of those made by earlier investigators whose reports would seem to have been dismissed as fiction. Birch (1859) using  $O_2$  therapeutically observed that occasionally strange reactions were precipitated by breathing  $O_2$  which he thought were not explainable on a psychological basis. He describes these as "a sense of constriction of forehead and temples, a feeling of weight over the centre of the parietal bones, and in the occiput, a rush of blood to the head, fullness, pain, or oppressive sensation in the nape of the neck and base of the brain, sudden faintness, palpitation of the heart, violent reflex movements in extremities affected with paralysis of voluntary motion, a state of unnatural excitement of the entire nervous and vascular systems, which has continued for successive days. The chief symptoms of a disturbing

character observed from pushing very large doses of the gas are, in thin anaemic persons, sudden or gradual disappearance of the pulse, pallor of countenance, coldness, and partial collapse, in the plethoric or sanguineous the reverse—viz, too-excited circulation, full bounding pulse, intense heat of head, face, and skin, severe, oppressive headache "

Smith (1870) likewise reported that breathing  $O_2$  sometimes produced subjective responses such as a sensation of freedom about the chest, a feeling of warmth below the sternum, occasionally a slight degree of vertigo, a disposition to yawn constantly during inhalation and an inclination to sleep Haldane (1917) reported that the immediate effect of suddenly administering high concentrations of  $O_2$  to a cyanosed person was sometimes unpleasant as he had observed from experiments on himself and others Under such conditions, he says, "The heart may become tumultuous in its action and the breathing irregular," and advised against too rapid addition of  $O_2$  to the inspired air in cyanosed patients DuBois (1928) remarks that the symptoms of anoxemia curiously enough "are sometimes exaggerated when the subject receives oxygen and not in frequently he acts like a drunken man "

The reports of untoward reactions to breathing  $O_2$  after exposure to low  $O_2$  or after a prolonged period of anoxemia in disease, recall also the experiments of Haldane and Smith (1893) in which they found that subjects after rebreathing air in a chamber for several hours to a point where the  $CO_2$  had reached 6 or 7 per cent and the  $O_2$  had fallen to about 13 per cent, were nauseated, vomited, and suffered headache *after* their return to room air Somewhat comparable effects were also noted in subjects after having been exposed to a gradual depletion of  $O_2$  where the  $CO_2$  was kept low

Alexander, Duff, Haldane, Ives and Renton (1939) found that return to normal air or to pure  $O_2$  after having been exposed to high  $CO_2$  (6 to 7 per cent) for an hour, resulted in similar disturbances It has been suggested (Haldane, 1941) that such nausea and vomiting may have been responsible for some deaths occurring in the use of submarine escape apparatus where the subject breathing a low  $O_2$ -high  $CO_2$  atmosphere is suddenly exposed to pure air or concentrated  $O_2$  The vomiting, he says, can be avoided by purifying the air, or by breathing  $O_2$  or pure air for some minutes before attempting escape, thus would give time for the vomiting to occur if it is going to do so

The fact that such disturbances occur only on the return to normal air or to increased  $O_2$  tension must represent a failure of the organism rapidly to readjust to the higher  $O_2$  tension But while such reactions are precipitated by the return to the increased  $O_2$  tension, they may perhaps be considered as the delayed effects of the  $CO_2$  or low  $O_2$ , rather than as adverse effects of the increased  $O_2$  tension itself The delay in the precipitation of the disturbances until after the return to normal air may then simply indicate that as a result of the gradual accumulation of  $CO_2$  and the depletion of  $O_2$ , medullary centers may have been depressed to a point where they become incapable of normal response, then with a rapid return to increased  $O_2$  tension the reactivity of those centers recovers before the offending chemical state of the cells has been completely rectified

The fatigue commonly experienced after shifting from low  $O_2$  to a higher  $O_2$

tension deserves special mention in this connection. The fatigue felt on the return to normal air or pure  $O_2$  after breathing low  $O_2$  as described by Haldane and Smith, (1893) is highly suggestive of a persistence of some functional disturbance or organic damage sustained during the low  $O_2$  exposure, the recovery from which is slow. Dill (1938), for example, points out that some of the C N S effects of low  $O_2$  may be caused by an increased intracranial pressure arising from an altered capillary permeability which as Landis (1928) has maintained occurs in  $O_2$  lack. It is difficult to see how complete recovery from such conditions could occur immediately on return to normal air. Dill's report emphasized the persistence of fatigue effects of low  $O_2$ , although Horvath, Dill and Corwin (1943) observed no lasting harmful effects on the C N S of schizophrenic patients after their exposure to anoxia severe enough to produce brief periods of unconsciousness.

In connection with the fatigue effects of low  $O_2$  it is of interest that Monge (1943) has emphasized again that sub-acute mountain sickness is a "fatigue disease" and that exposure to high altitudes may change the capacity of the tissues to utilize  $O_2$ . Monge et al (1928), Edwards (1936), and Barron, Dill, Edwards and Hurtado (1937) have also suggested that in low  $O_2$  exposures there is a failure in  $O_2$  utilization by the tissues, particularly those of the C N S and that this might be caused by a change in respiratory enzymes.

Such alterations in enzyme mechanisms, especially where they are not immediately reversible, might help to explain some of the reactions precipitated by a shift from low  $O_2$  to the higher  $O_2$  tensions of normal air or pure  $O_2$ , and are therefore pertinent to the consideration of those responses experienced following actual or simulated flights to high altitudes. Romano et al (1943) found that syncopal reactions persisted for some time after an individual's return to normal atmospheric pressure following simulated flights to high altitudes (35,000 ft) in decompression chambers. In some cases the severity of these reactions which continued for as long as 24 hours, actually increased rather than decreased when the higher  $O_2$  tension of normal air was readministered. The question arises as to just how far these syncopal reactions and the well recognized post flight fatigue represent failure of the organism rapidly to readjust to a sudden shift to an increased  $O_2$  tension after having been "acclimatized" to low  $O_2$  tensions. It has been claimed that post flight fatigue is due to "silent" aeroembolism (Behnke and Willmon, 1941, Behnke, 1942) but as Carson (1941) points out such an explanation is without substantial support. Certainly the evidently complex etiology of post flight reactions (MacFarland, 1941) make it appear very unlikely that they can be attributed to any one single factor alone, the enthusiasm with which aeroembolism was first grasped as the explanation for so many of the responses to high altitude conditions has not been justified by the experimental evidence.

Low  $O_2$  constitutes the predominant stimulator for the chemo-receptors of the carotid reflex structures (Gesell, 1940). A sudden increase in  $O_2$  tension diminishes or even abolishes the reflex drive arising in this structure, possibly as the result of a shift toward the alkaline side induced by the release of Na from the

lactate ion, now, if at the same time the sensitivity of the central regulators, the adequate stimulus for which is predominantly  $\text{CO}_2$  (Gesell, 1940) be depressed, for example by prolonged excess  $\text{CO}_2$ , low  $\text{O}_2$ , or anesthesia, breathing and other vital phenomena will be profoundly affected. Evidence that this is so finds support in the work of Moyer (1941) in which it was pointed out that if anesthesia be of such level as to depress or abolish the response to  $\text{CO}_2$ , the administration of increased  $\text{O}_2$  tension induces respiratory failure, and further that whenever artificial respiration is indicated during evipal or phenobarbital anesthesia, the gas mixture should not contain  $\text{O}_2$  in concentrations of more than 40 per cent.

c *Evidence of Adverse Effects of Increased Tensions of  $\text{O}_2$  from Exposures to Compressed Air at Moderate Pressures* Lorrain Smith (1899) was of the opinion that the increased  $\text{O}_2$  tension of compressed air might play a part in the production of caisson sickness because he found some of the pathology of  $\text{O}_2$  poisoning present also in caisson sickness. This suggestion was dismissed by subsequent investigators who had become justly convinced that caisson sickness was caused for the most part by the release of nitrogen bubbles on decompression. The deep diving and high pressure work of late years, however, has necessitated a reconsideration of  $\text{O}_2$  poisoning as a possible contributor to the reactions observed not only during the actual exposure to the increased air pressure, but also in the subsequent decompressions.

It has become increasingly evident that it is unsafe to assume that because an increased concentration of  $\text{O}_2$  at atmospheric pressure frequently exerts no apparent toxic action in short exposures, there would be a similar absence of effects with the same tension of  $\text{O}_2$  in increased air pressure, where other variables are involved. For example, there is experimental evidence that the presence of  $\text{CO}_2$  may be an important determinant in the action of  $\text{O}_2$ . Even a relatively slight increase in the  $\text{CO}_2$  tension, whether it be due to inadequate ventilation, an altered rate of diffusion or an upset in the  $\text{CO}_2$  carriage by the blood (see section II below) augments the toxic action of  $\text{O}_2$ . In addition to this there is the influence of the increased concentration of nitrogen.

It is entirely unlikely that the increased  $\text{O}_2$  tension in compressed normal air, even with the high pressures now frequently employed in deep diving, would of itself cause  $\text{O}_2$  convulsions, provided the  $\text{CO}_2$  were kept low and the exposure not unduly prolonged, but there is evidence that such increased tension of  $\text{O}_2$  can induce lung damage which may be partially or completely masked by the wide margin of safety provided in normal lungs. It has long been accepted (French, 1916, Haldane and Priestley, 1935, Fraser, 1940) that broncho-pneumonia following prolonged exposure to compressed air in deep diving has been caused by the increased  $\text{O}_2$  tension in the neighborhood of 760 mm Hg.

But besides causing lung damage,  $\text{O}_2$  in high concentration alters the fundamental oxidative mechanisms in the tissues and this, as is also true of the lung changes, is not always rapidly reversible. These possible  $\text{O}_2$  effects induced by compressed air, even though they be sub-symptomatic during the exposure, are worthy of consideration in relation to the problem of decompression, particularly since End (1938) claims, "An explanation of compressed air illness based entirely



upon theories of bubble formation is not altogether satisfactory," and an analysis of some of the experimental data presented by Behnke and Shaw (1937) concerning the use of  $O_2$  in the prevention of caisson sickness strongly suggests that the problem of decompression is not always simply one of eliminating the formation of nitrogen bubbles

The report of French (1916) illustrates the well known difficulty sometimes encountered in the safe decompression of divers but it too suggests that part of that difficulty may be attributed to incompletely or slowly reversible changes induced by prolonged exposure to increased  $O_2$  tension. Thus the conditions would approximate those obtaining in animals which, after adaptation or "acclimatization" to pure  $O_2$  at atmospheric pressure, show distressing symptoms when returned to normal air—symptoms which can be largely relieved by the animal's return to the  $O_2$  atmosphere. The almost immediate improvement which commonly results from recompression treatment in many cases of compressed air illness may be due, then, not simply to the dissolution of nitrogen bubbles, but also to the re-establishment of the abnormal environment which the "acclimatization" changes in the lung or in cellular oxidative processes require for tissue function. The inclusion of this possibility as a part of the explanation of decompression difficulties does not, of course, detract from the importance of the usually accepted interpretation, viz, that of nitrogen bubble formation.

The recompression chamber has become an indispensable adjunct to all deep diving operations and occasionally prolonged recompression treatments have been employed. Haldane and Priestley (1935) remarking on the difficulty on safe decompression from compressed air, say it may sometimes be necessary to keep the patient in the recompression chamber for 24 hours or more. These long recompression treatments offer further suggestive evidence of adverse effects of  $O_2$  on man especially since "acclimatization" to the increased  $O_2$  tension, unlike the disappearance of the nitrogen bubbles, will progress indefinitely so long as the high  $O_2$  tension is maintained. While recognizing the advantages of recompression treatment in compressed air illness, it would seem wise to keep in mind the possibility that prolonged exposure to the increased  $O_2$  tension in the recompression chamber may also involve the disadvantages of augmenting the  $O_2$  "acclimatization." This consideration is particularly apropos the use of pure  $O_2$  at increased pressure to facilitate the washing out of nitrogen from the body.

Some of the cases of Shilling, Hawkins, Polak and Hansen (1935) in which compressed air illness was treated by recompression, are highly suggestive that pathology was induced by the increased  $O_2$  concentration, for example, a diver suffering from compressed air illness was completely relieved by recompression to an air pressure of 75 pounds, but during the 294 minutes of the subsequent decompression he had a definite chill and began a dry unproductive cough which persisted after his removal from the chamber, his temperature was  $102.4^\circ F$ , pulse 108 and respiration rate 56 per minute. The x-ray examination showed irregular congestion in both lungs. The authors concluded "These findings are not typical of pneumonic consolidation but resemble more that of an irritation

Diagnosis of 'bronchitis, acute, condition considered to have been an irrita-

tive bronchitis' was made, and after nine days in the hospital condition had cleared sufficiently for discharge." Several factors may have been involved in the production of the pulmonary condition described, but the relatively prolonged exposure to an air pressure of 75 pounds which would be equivalent to 1.2 atmospheres of pure  $O_2$  together with the nature and time of the onset of the pulmonary symptoms make it more than probable that the increased  $O_2$  tension was an important contributor to, if not the underlying cause of, the "irritative bronchitis."

On the whole the evidence concerning the injurious effect of exposure of man to  $O_2$  in high concentration at atmospheric pressure is at present not very voluminous but there is sufficient to indicate that  $O_2$  does cause pulmonary damage, although there is a wide individual variation in susceptibility. Apparently no human lungs have as yet come to autopsy as a result of prolonged exposure to  $O_2$  at atmospheric pressure so that no fair comparison can be made of the pathology induced in man with that so well demonstrated in the lungs of experimental animals.

Those who have argued the innocuousness of breathing  $O_2$  in high concentrations have done so very largely because of their failure to observe any very pronounced symptoms of damage. It may be well, however, to emphasize the recognized fact that in most diseased conditions the initial pathology is sub-symptomatic and that not infrequently the pathology must extend beyond a wide margin of safety before symptoms are grossly manifest, certainly in the oxygen poisoning in experimental animals, lung pathology precedes, rather than follows, the occurrence of overt symptoms. This, as Paine et al (1941) suggest, may be one reason why  $O_2$  poisoning has not been observed more frequently in the therapeutic use of high  $O_2$  concentrations on man.

There would seem to be no question but that continuous breathing of pure  $O_2$  at atmospheric pressure for periods exceeding 20 hours is damaging to the human lung and that even for periods as short as 4 hours some individuals of greater susceptibility may show symptomatology of lung involvement. The danger of administering pure  $O_2$  in therapy may, however, be very largely mitigated by occasional interruption of the exposure, as indicated from the animal experiments of Soulie (1939) and Paine et al (1941). Obviously the use of pure  $O_2$  in higher altitudes is attended with much less likelihood of danger because of the lower barometric pressure.

In summary it may be said that notwithstanding the present exigencies and the implication in some reports that continuous breathing of pure  $O_2$  is innocuous, the accumulated evidence from animal experiments (including those on man) indicate that continuous administration of pure  $O_2$  at atmospheric pressure, even for periods of a few hours' is attended with danger of pulmonary damage in some individuals and therefore demands the exercise of caution.

In addition to possible pulmonary injury, the administration of increased tensions of  $O_2$ , particularly in shifts from environments of low  $O_2$ , high  $CO_2$ , or both, is not infrequently attended by adverse reactions which are not readily attributable to any lung injury or irritation induced by  $O_2$ . These reactions may be

variously interpreted (1) to an inadequacy in rapid adaptation to increased  $O_2$  tensions, possibly reflecting in final analysis a lag in the readjustment of enzyme mechanisms, (2) the persistence of physico-chemical states in the C N S which, built up gradually without gross manifestation during the exposure to low  $O_2$  or high  $CO_2$ , give rise to overt reactions on sudden shift to  $O_2$ , (3) the continuation of reactions induced by the low  $O_2$ , after the administration of increased  $O_2$  tension, (4) the action of increased  $O_2$  tension on specific regulatory mechanisms such as carotid reflex structures

The literature offers no justification for discarding the generalization that for continuous administration, the concentration of  $O_2$  should not be much, if any, above 60 per cent. Yet in some conditions 60 per cent may be too high even for short exposures, in others 100 per cent may be too low for the end desired. It would appear that each case might best be considered individually. Where the administration of high concentrations of  $O_2$  does seem desirable the administrator still must weigh the possible benefit to be derived, against the possible dangers to be incurred. Argument derived largely from observations on individuals possessed of wide margins of safety may be misleading but they cannot reverse the well-established sign posts which unquestionably point to the deleterious effects of the continuous administration of  $O_2$  in high concentrations.

II POSSIBLE COMPLICATING VARIABLES INTRODUCED BY COMPRESSED AIR OR ARTIFICIAL GAS MIXTURES. The evidence presented above leaves no doubt that the increased  $O_2$  tension of moderately compressed air exerts adverse effects on living organisms which in large part are essentially similar to those induced by equal  $O_2$  tensions in hyperoxygenated air at atmospheric pressure but the assumption that the only action of compressed air is that of its contained  $O_2$ , demands further consideration. This demand becomes particularly insistent not only in view of the fact that very highly compressed air has been used as a means of studying the effects of  $O_2$  at pressures above one atmosphere, but also because of the practical application of highly compressed air or artificial media in submarine and deep diving operations. An examination of the available data reveals that in some circumstances the effects of the increased  $O_2$  tension obtaining under increased pressures are inextricably interwoven with those of other factors.

*Effects of Increased Pressure Per Se.* The argument, so commonly advanced in many of the early reports, that pressure *per se* exerted physiological effects, were, for the most part, concerned with the vascular system. That viewpoint was expressed by Moxon (1881) who stated "There is one power by which blood can be sent into the vessels of the brain with certainty, and with any degree of force that may be desired, and that power is atmospheric pressure. It needs no experiment to show that great increase in atmospheric pressure must drive the blood away from the surface of the body and into any parts that are not accessible to air, such parts are the interior of the cranium and spinal cord." Essentially similar beliefs were expressed by numerous earlier authors (Babington and Cuthbert, 1863, Vivenot, 1865, 1868, and others).

This idea of the mechanical effects of pressure was also held by Smith (1886) who maintained that the beneficial effects to be derived from recompression

treatment in compressed air illness, was a more gradual release of the blood from the deeper tissues into which it had been shifted during the exposure to the compressed air in accordance with certain "laws," the first of which was, that "under high atmospheric pressure the centres will be congested at the expense of the periphery", a "second law" stated that "firm and compact structures will be congested at the expense of those more compressible", and a "third law" held that "structures within bony cavities are congested at the expense of all others". The blood, according to Smith, was "distributed not in accordance with physiological demands but in obedience to overpowering physical force". It was suggested that a similar congestion within bony cavities occurs during the barometric pressure alterations accompanying weather changes and Smith proposed that those persons who regard themselves as "walking hygrometers" and are accustomed to say they feel the dampness in their bones, are really "barometers perhaps quite as sensitive as the instrument of Torricelli." The experiments of Poiseuille (1835), of Loewy (1895) and Hill and Macleod (1902) and the observations of Bert (1878), and Hunter (1887), however, amply demonstrate the fallacy of the claims that in compressed air the blood is mechanically squeezed from the peripheral vessels by the direct mechanical effects of the increased air pressure.

Bert concluded that the blood pressure and the increased pulmonary capacity changes, induced by exposure of animals to moderately increased air pressure were entirely due to the mechanical effects of the increased air pressure acting on the diaphragm and abdomen. This conclusion is of especial interest since in another connection Bert insisted that the only effect of increased air pressure is that caused by the increased tension of the contained oxygen. DuBois-Reymond (1899) reported that exposure to compressed air tended to lower the diaphragm but Heller, Mager and von Schrotter found no evidence that the diaphragm was lowered or that the vital capacity was increased in compressed air. More recent investigations (Hill, 1912, Shilling, Hansen and Hawkins, 1935) indicate that the vital capacity of divers is increased in compressed air.

The report of Case and Haldane (1941b) indicates that articular surfaces and synovial fluid may be physically affected by increased air pressure. 'At high pressures loud cracking noises, audible to others, were frequently produced when J.B.S.H. moved his shoulder joints. Clearly the pressure between the articular surface is increased tenfold, and the effect of irregularities must be enhanced. No pain was associated with these sounds.' However, because of equal distribution of pressure throughout fluids it is a bit difficult to see just how bearing pressure between the articular surfaces could be increased to this degree, the explanation offered for the audible sounds would appear to necessitate the assumption of a compressional alteration in the volume of synovial fluid, or of gas which may have accumulated in the joint capsule during previous exposures to, and decompression from, high pressures. In any case if exposure to compressed air does increase the bearing pressure on the articular surfaces it is an exceedingly important mechanical effect of pressure.

Armstrong (1938) has maintained that because of the gases dissolved, a volume of water will be 'considerably' increased by a decrease in atmospheric pressure.

and that since fats dissolve five times as much nitrogen as water their volume should be even more profoundly altered by pressure changes. If, as is claimed, these volume changes are physiologically significant under reduced pressure, they might, conceivably, be significant also under increased pressures.

Ebbecke (1914) reported that increased hydrostatic pressure may of itself act as a stimulating factor in some conditions but that tissue can withstand pressures of 100 atmospheres without injury. Edwards and Cattell (1928) found alterations in the contractions of cardiac muscle when exposed to increased hydrostatic pressure and believed that the stimulating effect of pressures between 45 and 80 atmospheres is related to fundamental changes in the physical system of the tissue.

Further evidence that high pressure causes alterations in living cells is found in the work of Brown (1934). Ebbecke (1935a, 1936) regarded the paralysis caused by high pressure as a mechano-narcosis and thought it was in some respects comparable to electro-narcosis. Increased thigmotaxis and geotaxis and a tendency to rolling movements were induced in paramecium. In other work (Ebbecke and Shaefer, 1935) the action potentials of muscle and nerve were found to be altered, the excitability of nerve was increased and rhythmic responses were elicited by a single stimulus applied under the high pressure. Grundfest (1936) also reported alterations in the reaction of nerve fibre and Brown (1936) described changes in the isometric twitch under high hydrostatic pressure. Marsland and Brown (1936) and Brown and Marsland (1936) and Marsland (1939) report effects of hydrostatic pressure on ameboid movement.

Deuticke and Ebbecke (1937) discussed the chemical and physico-chemical alterations occurring in tissue exposed to high pressure. Ebbecke (1938) found that exposure to increased pressure caused frog erythrocytes to change from plate to sphere form and although the volume was unaltered thereby, there were accompanying intracellular changes.

Cattell (1936) in review of the subject of biological effects of high pressures has pointed out that it is well-nigh impossible correctly to evaluate the effects of pressure *per se* on living organisms by the use of compressible gas media because of the concomitant alteration in gaseous tension. This complicating factor may be largely, if not entirely, obviated by the use of hydraulic media and the results of experiments using hydrostatic pressure indicate that the responses of tissue may be altered by very high pressures. Fontane (1929a,b) in experiments involving pressures ranging from about 25 to 150 kgm per sq cm, found that the metabolism of fish (as determined by  $O_2$  consumption) was augmented by pressures up to about 100–125 kgm per sq cm, at higher pressures it was diminished. Because this pressure range is not so very far from those encountered in deep diving it would appear unwise to hold categorically that in deep diving the high pressure *per se* exerts no effects whatsoever on physiological processes. On the whole, however, the evidence derived from hydrostatic pressure experiments indicates that those pressures which man has thus far encountered in diving operations do not demonstrably affect vital processes of the tissues themselves.

Increased pressure appreciably alters viscosity of gases and their diffusion, and

on the basis of these physical changes Thompson (1889) offered explanations for some of the effects he observed in animals exposed to compressed air, Hill and Macleod (1903) likewise pointed to these physical changes as possible contributing factors. One interesting manifestation of the effect of the increased density of the compressed air is the observation reported by Triger (1841) that air pressures of about 3 atmospheres caused an inability to whistle and imparted to the voice a peculiar nasal quality, pain in the ears relieved by swallowing was also noted. Similar observations have been noted commonly since that time (Bucquoy, 1861, Pol and Watelle, 1854, Foley, 1863, Hill and Macleod, 1903, Hill and Greenwood, 1906, Case and Haldane, 1941b). Smith (1886) in contrast to Triger (1841) reported that the sensitivity of hearing was diminished in compressed air and attributed this to the damping effect of the increased density on the functional vibration in the auditory apparatus. Interestingly enough Foley (1863) observed that his watch consistently lost time when in compressed air of a caisson, and this he interpreted as a result of the increased resistance offered by the compressed air to the swing of the balance wheel.

The report of Case and Haldane (1941b) presents evidence of the importance of the increased density of highly compressed air in respiration. At ten atmospheres the work of breathing, they say, is more than ten times greater than at normal pressure and with some types of canisters this increased resistance to breathing in compressed air was found to be "unbearable." Because of this, together with the coughing (attributed to caustic dust stirred up from the respirators by the increased turbulence in compressed air) and the lack of self control induced by the increased pressure, subjects removed their respirators when they should not have done so. The authors recommended that because of these factors all respiratory apparatus intended for use at high pressures should be tested under service conditions rather than at normal atmospheric pressure. This recommendation emphasizes the danger of assuming that the significance of a given mechanical or chemical factor in the functioning of respiratory apparatus is constant at both high and normal pressure, it would appear to be equally if not more dangerous to make similar assumptions concerning the physiological respiratory apparatus within the mammalian organism. Further mention of the physical factors of viscosity and diffusion is made below in connection with the effects of nitrogen.

The mechanical effects of increased pressure are obviously of importance where there is an absence of the normal patency of orifices leading to air pockets within the body. Smith (1886) reported that pain and other subjective responses arose from the frontal and maxillary sinuses during exposure to increased pressure because of temporary or partial blockage of the normal passages. Lack of adequate pressure equalization within such pockets may then elicit responses involving not only autonomic mechanisms but also the organism as a whole. Moreover, because the degree of patency may vary under different conditions, the magnitude of the response arising from this source is not uniformly predictable. In normal individuals trained to equalize the middle ear pressure Case and Haldane (1941) found that rapid compression, e.g., from one to seven atmospheres in 90 seconds

may be accomplished without any after effects or discomfort This rate of pressure increase, about one-half an atmosphere in 7.5 seconds, it was pointed out, corresponds to a vertical dive in air at a rate of about 1600 miles per hour

These same authors (Case and Haldane, 1941) did observe, however, that both pulse rate and systolic blood pressure rose in some instances and fell in others during rapid compression in air, but these changes did not appear to be related to the peculiar subjective sensations experienced on exposure to the high pressures Carlson, Ivy, Krasno and Andrews (1942) found no significant changes in blood pressure, respiration or heart rate of dogs as a result of subjecting them to very rapid air pressure changes such as encountered by aviation personnel Shilling and Willgrube (1937) state, "It is well known that if pressure is applied too quickly the diver becomes dizzy and often so dazed as to require several minutes to orient himself" The rapidity of onset of this effect suggested to them that some mechanical compression factor might possibly be responsible for this subjective effect

The question of the significance of intestinal gas pockets does not assume the importance to the diver that it does to the high altitude flyer yet in some cases where considerable gas is present at atmospheric pressure, rapid compressional reduction of intestinal gas volume may affect the organism although perhaps not adversely Snell (1896) remarked that workmen are in the habit of tightening their belts after entering the compressed air in caissons and he interpreted this as a response occasioned by the decreased volume of intestinal gas caused by the increased pressure Incidentally, the increased volume of intestinal gas which occurs on decompression from compressed air seems to have attracted little attention although it would appear to be worthy of consideration as a possible contributor to decompressional effects

The problem of pressure equalization in air pockets may, under some circumstances, also involve the lungs directly Some indication of this is found in the report of Case and Haldane (1941b) One of their subjects suffered a pneumothorax as a result of three exposures to compressed air at 8.6 atmospheres The x-ray examination, in addition to showing pneumothorax on the left side, revealed what appeared to be "emphysematous bullae at the extreme right apex" There was no evidence of tuberculosis The thoroscopy 23 days after the last experiment showed the left lung was almost entirely collapsed After a slow recovery there were two relapses in eight months Although the subject was an athlete the pneumothorax was thought to have been due to the presence of congenitally weak areas in the lungs The immediate cause of the rupture was not determined but several possible explanations were offered, viz, (a) "The lung was over-distended while inflating the Eustachian tubes during compression, (b) The subject held his breath during a decompression, and the expansion of the air brought about the rupture", (c) improper manipulation of valves of rebreathing apparatus, causing a sudden increase in gas pressure in the lung, (d) "A bulla with poor communication with the bronchi existed and filled up with air at high pressure During decompression the air could not escape into the bronchi, and burst out into the pleura"

The experiments of Polak and Adams (1932) and of Adams and Polak (1933) would seem to leave little doubt but that fatalities which occurred in the use of the submarine escape apparatus in relatively shallow water (about 15 ft) were the result of lung injury caused by the undue pulmonary distention while holding the breath during the ascent. In the light of these experimental findings, the most likely of the explanations offered by Case and Haldane for their pneumothorax mentioned above are (b) and (d). The probability of distending the lung during compression would appear to be much less than during decompression when the breath is held, unless of course some bronchi and bronchioles leading into air containing alveoli were plugged so that equalization of the alveolar pressure could be attained only by excessive stretch or rupture of adjacent alveoli in free communication with the trachea. If it be admitted that distention of the lungs, either in whole or in part, may occur on compression, one must then recognize the possibility that influences other than that of pulmonary rupture may arise from the lungs distended to points short of rupture, and that such influences may very well contribute to the responses which occur on exposure to compressed air.

This is especially pertinent to those subjective and neuro-muscular reactions which appear immediately or within a very few minutes after compression. An unequaled increase in intrapulmonary pressure may not only profoundly affect breathing but as the experiments of Luckhardt and Johnson (1928) showed, it may also affect the heart, reflexly, and by direct central asphyxia, and it may abolish the knee jerk and render the animal unconscious and anesthetized in 12 seconds.

It has also been found that increased intrapulmonic pressure alters the response of striated muscle to electrical stimulation of its motor nerve, although it has no evident effect on contraction elicited by direct stimulation of the muscle (Bean, 1942), from this it would appear that increased intrapulmonary pressure and lung distention influence the mobilization of the contractile response through some action on the neuromyal junction. Such reactions arising from pulmonary distention are more than of passing interest in connection with the submarine escape fatalities referred to above, for with the lung distention which is said to have occurred under those conditions, the resulting circulatory, cortical and neuromuscular reactions may have rendered the subjects unconscious or completely incapable of voluntarily controlling their breathing and muscular activity even in the first few seconds of the ascent to the surface.

Walsh (1941) in observations on a patient in whom there remained extensive defect in the skull following recovery from a right temporal craniotomy, found that exposure to decreased pressure (barometric pressure 247 mm Hg and pure  $O_2$  as the respiratory gas) caused an elevation of the scalp 1 cm. above its pre-exposure level and that return to atmospheric pressure caused a return to its original position. Exposure to increased pressure, on the other hand caused a reversible depression to 1 cm. below the level of the skull. These results were interpreted as indicating that these pressure changes had induced an alteration in the volume of cranial contents because of the pressure effects of air contained



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especially since some of the less reliable may have been unduly tinged with the enthusiasm of over zealous oxygen or compressed air therapists or their antagonists. But recent observations, amounting to a rediscovery of the subjective and neuromuscular reactions to compressed air, elevate many of those older claims, previously dismissed as incredible, to a position of validity little below that of the current reports.

Damant (1930) reported that some divers became abnormal mentally and suffered a loss of memory in deep dives (300 ft). He also pointed out that experienced diving officers have long recognized that subtle changes in character and behavior sometimes occur in divers even at lower air pressures. Phillips (1931) presented more detailed information concerning these subjective and mental responses in deep diving. In going to depths of from 270 to 300 feet, divers "found that it was much more difficult to assimilate facts and exercise the quick decision essential for successful diving. It might be summed up as a slowing of cerebration." Some divers said that they had "passed out" when on the bottom and others felt as though they were "under an anaesthetic." Hill and Phillips (1932) and Thompson (1935) remark further on these subjective effects of increased pressure. Behnke et al (1935) reported that in laboratory workers exposed in a chamber to air pressures as low as 4 atmospheres, there occurred emotional and neuromuscular disturbances which seriously interfered with accurate performance of simple routines in laboratory technique, no objective or subjective tests were carried out but it was maintained that these effects of compressed air are "immediate in their onset."

Shilling and Willgrube (1937) state that 5 atmospheres of air pressure induce a feeling which is likened to drunkenness. They are in essential agreement with Phillips (1931) and Hill and Phillips (1932) that there is manifest a dangerous overconfidence, accompanied by a dulling of mental ability, difficulty of assimilating facts and of making quick and accurate decisions. These authors (Shilling and Willgrube, 1937) present quantitative experimental data attesting to the slowed reaction time in mental and neuromuscular responses. They find also that increased experience materially lessens the subjective effects and that men with high mental ability do not fail as quickly as do those of lower intelligence.

Case and Haldane (1941b) in an interesting and informative series of experiments on themselves and other human subjects, found that air pressure at 5 atmospheres caused no noticeable subjective or objective changes and even at 7 atmospheres there was no deterioration of mental ability, arithmetic problems actually being performed more rapidly and with fewer mistakes. But at 8.6 atmospheres a variety of subjective reactions were observed, e.g., two individuals were slightly confused, two others were distressed and felt as if they were going to faint, one was euphoric and felt very confident, and another was mildly elated. Still another was unusually obstinate but showed no obvious emotional reaction. At 10 atmospheres two subjects were "somewhat euphoric on the first occasion, but later this wore off", another "varied between depression and elation." At 10 atmospheres the changes were quite definite, many of the subjects reported they felt drunk. Strange sensations were felt on the lips "something like velvet.

The timing of tests, making notes and gas sampling were either not carried out or were performed only with great difficulty, and some observations made under these conditions were not very satisfactory "It is quite imperative," these authors say, "that no great trust should be placed in human intelligence under these circumstances" The variety of effects described clearly indicates that there is no *one* subjective response which characterizes the intoxicating action of compressed air, but rather that the subjective reactions may vary widely in different individuals

In contrast to statements of Behnke and Yarbrough (1938) Case and Haldane (1941b) maintain, "It is quite incorrect to say that people are stupefied at such pressures However, they are definitely less responsible than when normal" The latter authors also found that the symptoms reached a maximum within 2 minutes of compression and did not increase after 30 minutes at 10 atmospheres It was thought there might have been a "slight degree of habituation" but this, it was admitted, might have been purely psychological The higher intellectual functions of the brain were apparently more profoundly affected than those involving muscular skill Decompression to 5 atmospheres almost always caused an immediate feeling of subjective improvement

Air at 8 atmospheres was found to taste "harsh, metallic and indefinable" (Case and Haldane, 1941a), and this was thought to be the taste of the concentrated nitrogen rather than of the  $O_2$  Green (1861) had apparently experienced a similar taste at much lower air pressure but explained it thus "The pumps which were used for forcing air into the armor were of brass and with each movement of the pump I could plainly taste and smell the copper in the atmosphere I breathed" In view of the fact that subjective and sensory changes and impaired judgment occur at increased pressures, one may perhaps question whether these gustatory effects reported by Case and Haldane and by Green may not represent disturbances in the more central parts of the sensorium rather than true sensibility arising from receptor stimulation by the increased concentration of the compressed gas

*Proposed Explanations for the Subjective and Neuromuscular Reactions to Compressed Air* Various explanations have been offered for these subjective and neuromuscular reactions experienced in exposures to compressed air sometimes referred to as compressed air intoxication, one of the earliest of these was that the increased  $O_2$  tension was the cause The rediscovery and current re-emphasis of the importance of these reactions, has brought forth several newer explanations, some authors have held that they are of psychological origin and others have concluded that they are due solely to a narcotic action of nitrogen An inspection of the data reveals that none of these is in itself an entirely satisfactory explanation of the phenomena and that additional etiological factors must be considered

1 *Increased  $O_2$  tension as the cause of the reactions to compressed air* Tiger (1841) pointed out that physical exertion could be carried out with less breathlessness and discomfort in compressed air than in air at ordinary pressure Furthermore the subjective effects of compressed air such as the feeling of invigoration and well being were claimed by therapists to be the same as those

induced by breathing  $O_2$  and it was therefore claimed by many (Demarquay, 1866) that the effects of compressed air were simply those of the increased  $O_2$  tension. Birch (1859) reported that breathing  $O_2$  at atmospheric pressure caused subjective and objective reactions in some individuals and maintained that these were not of psychological origin.

Bert (1878) provided more substantial support to the belief that the effects of compressed air were due to the increased partial pressure of  $O_2$ . He made the generalization that the effects of breathing air at 5, 10 and 15 atmospheres were the same as those induced by breathing  $O_2$  at 1, 2 and 3 atmospheres respectively, and having convinced himself of the verity of this generalization, utilized compressed air to attain the desired high  $O_2$  tensions in his studies on  $O_2$  poisoning. Since Bert's time, the assumption that the responses to compressed air (barring, of course, those of decompression) are caused only by the increased  $O_2$  tension, has been rather widely accepted (Bornstein and Stroink, 1912, Achard, Binet and LeBlanc, 1927, Binger, Faulkner and Moore, 1927, Cleveland, 1925, Smith, Heim, Thomson and Drinker, 1932).

Hill and Macleod (1903c), however, made the important observation that the effects of exposure to compressed air are not the same, but are actually more damaging than those elicited by pure  $O_2$  or by an equal partial pressure of  $O_2$  at atmospheric pressure. Similar findings have been reported by Orzechowski and Holste (1938). These results, contrary to the conclusion of Bert, are particularly noteworthy because they mean, of course, that the presence of nitrogen, the rare gases, or some physical or chemical feature of compressed air either enhances the deleterious effects of  $O_2$  or contributes in an additive manner to those effects.

The rate of change in the environmental  $O_2$  tension may have some etiological significance in the occurrence of the reactions of compressed air intoxication. Certainly if the shift from low  $O_2$  or normal air, to high  $O_2$  at atmospheric pressure elicits subjective and neuromuscular reactions (see preceding section), there would seem to be no good reason to exclude the possibility that somewhat similar reactions might occur as a result of a sudden shift from the low  $O_2$  or normal air at atmospheric pressure to the high  $O_2$  tension of highly compressed air, indeed, the work of Hill and Macleod (1903c) would justify the expectation that such reactions induced by a sudden shift to increased  $O_2$  tension might be augmented by the association with compression.

Damant (1930) was of the opinion that the subjective changes experienced by divers at depths of 300 feet were attributable to the increased partial pressure of  $O_2$ .

## 2 *Psychological factors as the cause of the subjective and neuromuscular reactions*

The subjective character of the reactions to compressed air suggests the possible involvement of psychological factors in their etiology and several references do point to the probable importance of such factors. That psychological and emotional influences were operative in the experiments with air pressures of about 60 pounds carried out by Hill and Greenwood (1906) is implied by their statement of having felt a "false sense of anaesthesia" and by the written comments of one

subject that he was "very nervous all through the experiment" and that there were "feelings of nervousness at being exposed to so high a pressure (which at times was somewhat acute especially when we were not engaged in analytical work) "

Phillips (1931) studied the responses to compressed air in some detail by psycho-analytical methods. This investigation uncovered considerable evidence that the mental stability of the individual played an important part in determining his reaction to the increased air pressures met with in diving. It was found that those men who had experienced untoward reactions either in deep or shallow water were "of the suppressed types, who habitually exercise control. Shy, reticent, and self-contained, they work best by themselves and do not relish observation. They are usually of a philosophic rather than a practical disposition." Phillips maintained that the cause of the divers' failure was claustrophobia. The importance of psychological elements is further suggested by the fact that some authorities have indicated that in the selection of men for deep diving, very complete psycho-analyses should be made.

One of Phillips' subjects, in commenting on the response to compressed air in an experimental chamber and that experienced in actual diving, said, "You cannot possibly compare the two conditions, in London in the chamber it was light and there were others with me, on the bottom it is dark and lonely." This statement is particularly arresting, not only because it points to the significance of psychological factors in the reaction to compressed air, but also because it emphasizes the fact, so commonly ignored, that the physico-chemical adjustments of psychological origin obtaining in the organism, and upon which the effects of the increased gas tension are superimposed, are by no means always the same in the experimental chamber as they are under actual diving conditions.

Hill and Phillips (1932) also indicate that claustrophobia is the cause of the subjective disturbances met with in diving. End (1937) likewise points to this as a factor of some importance. Thompson (1935) accepts the view that these reactions are of psychological origin since, as the diving trials continued, the reactions were less intense. Similar observations of Shilling and Willgrube (1937) that training diminishes the intensity of the response and that men of high mentality do not fail as quickly as those of lower intelligence further suggests the involvement of psychological factors. The report of Fraser (1940) emphasizes that "a nervous disposition" or "nervous instability" disqualifies an individual for deep diving because these are "bound to crop up in prolonged work, especially in deep water." The observations of Case and Haldane (1941b) that on a first compression the change in consciousness is very striking and "alarms some people" but that "when it is taken for granted it is likely to have less effect on behaviour," and that there might have been some "slight degree of habituation" to the effects, or that such an impression of habituation may have been "purely psychological," attest again to the possible significance of psychological factors. Mosso's investigations led him to the conclusion that apprehension so affects the nervous system that it "aggravates in an unexpected way" the influence of changes in barometric pressure, the data concerning the effects of compressed air

seem to warrant the extension of that conclusion to include not only low barometric pressure, but also increased barometric pressure

It would appear then that psychological factors cannot be completely dismissed in a consideration of the possible contributing causes to the subjective and neuromuscular disturbances experienced in exposure to highly compressed air, for psychological changes, which, in themselves may not be grossly manifest, might yet contribute indirectly to the effects of compressed air by providing a background of a peculiar milieu or physico-chemical adjustment which enhances or modifies the reaction to the more immediately responsible agents which are superimposed upon it

3 *Nitrogen as an etiological factor* It was inferred by Behnke, Thomson and Motley (1935) that the subjective and neuromuscular disturbances induced by exposures to compressed air were caused by nitrogen which at pressures as low as 3 atmospheres was said to act as a narcotic. The subsequent implied acceptance of this inference as fact, calls for a consideration of the experiments and argument upon which it was based

An examination of the data presented as evidence of nitrogen narcosis reveals it to be unconvincing for a number of reasons, the first of these concerns the reaction selected as the criterion of narcosis. It was reported (Behnke, Thomson and Motley, 1935) that euphoria was experienced by a group of laboratory technicians working in compressed air at pressures of 3 atmospheres, and since no euphoria was observed in earlier experiments on subjects exposed to pure  $O_2$  at atmospheric and higher pressures, it was inferred that the euphoria experienced in compressed air could not have been due to the increased  $O_2$  tension and that it must therefore have been caused by the nitrogen acting as a narcotic. The report of other authors (Case and Haldane, 1941), however, clearly indicates that in the psychological response to compressed air there are wide individual variations and that while euphoria does occur, there are equally unimpressive states of depression. Moreover, breathing pure  $O_2$  has been repeatedly reported, particularly in the older literature (Demarquay, 1866), to give rise to a sense of elation and euphoria. The presence of euphoria, therefore, does not appear to be a reliable differential symptom or an adequate criterion of narcosis

In the second place, the evidence of Behnke et al is unconvincing because the experimental subjects exposed to the increased  $O_2$  tension and who failed to experience euphoria under those conditions, were evidently not the same as those exposed to the compressed air. In the third place, the experimental conditions were not at all comparable. The subjects exposed to increased  $O_2$  tension wore masks or diving helmets during the several hours of each exposure, whereas those exposed to compressed air wore no mask or helmet and were free to move about while performing routine laboratory techniques in the company of their associates. It would seem that where such an intangible criterion as euphoria is concerned, all experimental conditions must be rigorously controlled before any inference can safely be drawn from the results

A conclusion that the subjective and neuromuscular reactions to compressed air are caused by a narcotic action of nitrogen obviously demands the rigid exclusion

of other possible causes. Now in order to rule out increased  $O_2$  tension as the possible cause of, or contributor to, the reactions in question, it has been assumed that the reactions to a given tension of  $O_2$  in compressed air are no different from those induced by the same  $O_2$  tension at atmospheric pressure. Such an assumption is based on the premise that an identical state of the tissues obtains under each of these two experimental conditions. But if the nitrogen in compressed air is a narcotic, how can the state of the tissues exposed to compressed air even remotely approximate that which obtains in an environment in which there is an equal  $O_2$  tension but an absence of nitrogen? Comparable tissue states are not provided in these two situations and the possible involvement of  $O_2$  as an etiological factor is therefore not satisfactorily ruled out. The argument of Behnke, Thomson and Motley (1935) appears then to be one in which the final inference or conclusion that nitrogen is a narcotic, disproves an essential premise of identical experimental conditions upon which that argument must be based.

The experiments of Hill and Macleod (1903c) as already mentioned, do indicate that the effects of compressed air are not simply those of the increased  $O_2$  tension alone. Compressed air was found to be more damaging than those of an equivalent  $O_2$  tension in the absence of compressed air, but even such difference does not justify a conclusion that the greater damage in compressed air is caused by nitrogen.

Another feature of the argument presented as supporting evidence of "nitrogen narcosis" was that the effects of compressed air, unlike those of increased  $O_2$  pressure, were immediate in their onset and did not become progressively more severe as the exposure was continued. This does lend some substantiation to the view that the effects of compressed air are not simply those of increased  $O_2$  tension, but like the results of Hill and Macleod (1903c) it provides no evidence that nitrogen at increased pressure is a narcotic. In fact, as Shilling and Willgrube (1937) have pointed out, the immediacy of the onset of the pressure effects and the absence of any increase in their severity as exposure is prolonged, constitute rather strong arguments against a narcotic action of nitrogen.

While the evidence up to this point fully justifies a conclusion that the reactions to compressed air are not simply those of the equivalent  $O_2$  tension at atmospheric pressure, and that compressed air must involve some factor of physiological importance other than the increased  $O_2$  tension, it offers no substantial support for the interpretation that the compressed air reactions in question are caused by the increased tension of nitrogen or that nitrogen at these tensions acts as a narcotic. Shilling and Willgrube (1937) pointed out that "the true cause of the slowed mental and neuromuscular activity encountered in high air pressure work has not been satisfactorily demonstrated."

The experimental work with helium, however, does provide some suggestive evidence that nitrogen contributes to the production of the subjective and neuromuscular reactions of compressed air intoxication. In 1919 it was suggested (Thomson, 1927) that helium, because of its physical properties, might be used to advantage in diving. At about the same time Cooke applied for, and was later (1923) granted a U S Patent for a method of using helium in pressure work (Yant, 1927). Since the experimental work of Sayers, Yant and Hildebrand (1925) and

the granting of a patent to these investigators in 1927, little or no practical application of helium in pressure work was made until after the test demonstration carried out under actual diving conditions by End (1937) and his collaborators. Its use then became more widely advocated (Behnke and Willmon, 1939, 1940, Fraser, 1940). The chief benefit claimed for the use of helium was that it permitted a very rapid decompression, although Case and Haldane (1941) not only reported that at pressures of ten atmospheres the use of helium failed to prevent bends but also pointed out that because of its solubility characteristics it is erroneous to expect that it should. "It is certain," these authors say, "that a mixture of this kind [85 per cent He and 15 per cent O<sub>2</sub>] cannot be regarded as superior to air as a prophylactic against bends."

But in addition to the facilitation of decompression resulting from the use of helium, End (1937) reported that the subjective and neuromuscular disturbances experienced in highly compressed air were absent in those exposures in which helium was substituted for the nitrogen of the respired air. Similar observations were made by Behnke and Yarbrough (1938). These results were highly suggestive that nitrogen was somehow causally involved in the subjective effects induced by compressed air.

The experiments of Case and Haldane (1941b) using O<sub>2</sub>-N<sub>2</sub> mixtures at pressures of about ten atmospheres demonstrate that the subjective effects of compressed air may occur even when the O<sub>2</sub> partial pressure is reduced to or even below the normal 20 per cent of one atmosphere. Convincing evidence is thus provided that an increased O<sub>2</sub> tension is not essential to the occurrence of the subjective disturbances experienced in compressed air. The experiments in which not only He-O<sub>2</sub> but also H<sub>2</sub>-O<sub>2</sub> mixtures were used, indicate that the presence of nitrogen contributes directly or indirectly and in large measure, to the occurrence of those disturbances. The authors interpret their data to mean that the intoxicating influence of air at high pressures is the result of a direct effect of nitrogen on the brain itself and thereby subscribe to the "nitrogen narcosis" hypothesis. They maintain, however, that this narcotizing action of nitrogen cannot be adequately explained on the basis of its chemical properties or its lipid solubility and suggest that intra- or extra-cellular adsorption processes may be the explanation of the direct nitrogen effects.

The experimental data at this stage conclusively show that the subjective and neuromuscular reactions to compressed air cannot be attributed to increased O<sub>2</sub> alone, that these reactions may even occur in the absence of an increased O<sub>2</sub> tension, and that the presence of nitrogen at increased pressure contributes to the occurrence of these reactions. But the interpretation that nitrogen of itself acts as a narcotic still awaits proof, other factors which might contribute to the precipitation of the compressed air reactions have not as yet been satisfactorily ruled out. It would appear, therefore, that until further and more substantial evidence is presented which indicates otherwise, "nitrogen narcosis" would best be considered a hypothesis. On the other hand, there are now a goodly number of indicators which point to another, and perhaps more plausible, explanation for the reactions to compressed air than that of "nitrogen narcosis."

4. *Carbon dioxide as an etiological contributor* Although Hill and Phillips



(1932), convinced of the importance of psychic factors, dismissed both  $O_2$  and increased  $CO_2$  as possible etiological elements in these peculiar compressed air effects, a consideration of the accumulated evidence reveals there is a very high probability that  $CO_2$  is causally involved and to such an extent as to render resorting to the "nitrogen narcosis" hypothesis for an explanation, quite unnecessary. One of the more significant reports in this connection is that of Behnke and Willmon (1939). These authors were unable to distinguish the effects of breathing increased  $CO_2$  by their subjects when exposed to increased air pressure, from those of "nitrogen narcosis". In fact they found that increased  $CO_2$  simply increased the intensity of the "nitrogen narcosis," and said "While we were aware of the symptoms of nitrogen narcosis at a depth of 240 feet, we were surprised at their intensity. For the application of pressure in a chamber equivalent to a depth of 240 feet elicits reactions of considerably lessened severity."

"Additional diving tests indicated that the difference in reactions between chamber and deep-sea diving could be attributed to the increase in carbon dioxide concentration in the diver's helmet."

"The symptoms, however, were not typical of high carbon dioxide tension in the lungs but rather of air at a depth of 300 feet or more. Increased depth of respiration, for example, did not precede loss of consciousness."

"Apparently the increase in carbon dioxide augmented the narcotic action of nitrogen."

These observations recall the great emphasis which Snell (1896) placed on the importance of the removal of  $CO_2$  from caissons by very thorough ventilation and the advice of DuBois (1928) concerning  $CO_2$  removal in diving operations. Davis (1935) has also stressed the dangers of even very small amounts of  $CO_2$  under such conditions.

It is generally recognized that a large excess of carbon dioxide may diminish rather than augment breathing. An absence of an increased depth of respiration cited by Behnke and Willmon (1939), then, provides no justification for dismissing the possibility that the response in question could have been caused by the increased  $CO_2$  especially in the presence of other variables, for it is widely agreed that the reaction to  $CO_2$  may be modified by a variety of conditions, particularly by an increased  $O_2$  tension (Bert, 1878, Hill and Flack, 1908, Gesell, 1923, Shaw et al, 1934, Haldane and Priestley, 1935, Case and Haldane, 1941). The observation that under some conditions increased  $CO_2$  tension in the presence of increased  $O_2$  tension not only fails to increase breathing but actually decreases it (Marshall and Rosenfeld, 1936, Dripps and Dumke, 1943), is of interest in this connection. It would appear, therefore, to be quite erroneous, and in some cases even dangerous, to assume that the response to a given  $CO_2$  increase in highly compressed air should be the same as that induced by an identical  $CO_2$  increase in air at normal pressure.

According to Haldane and Priestley (1935) excess  $CO_2$  even at atmospheric pressure in addition to causing ataxia, stupefaction, anesthesia and loss of consciousness has a "narcotic effect" which "quiets down respiration". The narcotic and anesthetic effects of carbon dioxide were noted and remarked upon by

several earlier investigators (Ewart, 1794, Beddoes and Watt, 1796, Bert, 1878, Foy, 1889, 1892, Hill and Flack, 1908), and Simpson (1872), aware of its reputed effects, considered the use of  $\text{CO}_2$  as a practical anesthetic. Wolff, Cobb and Fremont-Smith (1931) too, have emphasized the narcotic action of  $\text{CO}_2$  and believe that some of the effects of administering an  $\text{O}_2$ - $\text{CO}_2$  mixture to psychiatric patients may be caused by such narcotic action. The reports of Gellhorn and Spiesman (1935a,b) that  $\text{CO}_2$  causes auditory and visual disturbances also suggest that sensory changes which occur in compressed air could possibly be caused by an increased  $\text{CO}_2$  tension.

Barcroft (1938) reported that breathing high concentrations of  $\text{CO}_2$  at atmospheric pressure caused subjective and neuromuscular changes referable to an action on the highest part of the C.N.S., mental changes, and errors in manipulations requiring nicety of co-ordination such as the taking of gas samples. "Now the interesting point," he says, "is not that these errors occurred, though that is quite significant, but that I could have gone into a court of law and sworn that one at least of the two [samples] was correctly taken. When I came out I was retaining my grip of things only with an effort." The similarity between these effects and those attributed to "nitrogen narcosis" appears to be more than coincidental.

Although Case and Haldane (1941b) found that the intoxicating effect of compressed air was enhanced by the addition of  $\text{CO}_2$  and stated that the combined effects of high partial pressure of  $\text{N}_2$  and  $\text{CO}_2$  were much more severe than those of either alone, they had no explanation as to "why  $\text{CO}_2$  and  $\text{N}_2$  excess appear to co-operate." These observations point again to the possible identity of "nitrogen narcosis" and the effects of excess  $\text{CO}_2$ . The authors noted further that when the partial pressure of  $\text{CO}_2$  in compressed air rose above 3 per cent of an atmosphere, breathing was increased, but any subjective distress caused by breathing still higher partial pressures was much less than that at atmospheric pressure. This, the authors suggested, might have been due to the narcotic effect of nitrogen, but in view of the fact that excess  $\text{CO}_2$  may, under some circumstances, diminish breathing and that it exerts a narcotic influence, it appears unnecessary to resort to an hypothetical "nitrogen narcosis" for an explanation.

The experiments of Case and Haldane (1941b) again illustrate the fallacy of assuming that the effects of a given tension of  $\text{CO}_2$  in compressed air are always quantitatively and qualitatively the same as those induced at atmospheric pressure. The increased potency of  $\text{CO}_2$  acting in compressed air was demonstrated by the finding that air at 10 atmospheres containing 0.4 per cent  $\text{CO}_2$  (i.e., a partial pressure equivalent of 4 per cent of one atmosphere) caused marked deterioration in manual dexterity and mental confusion, in concentrations of from 6.6 to 9.7 per cent of an atmosphere, it caused a loss of consciousness in from one to five minutes. Significantly enough this loss of consciousness "in almost all cases took place quietly and easily," indicating again that it is erroneous to expect that excess  $\text{CO}_2$  in compressed air should invariably cause hyperpnea. At atmospheric pressure, on the other hand, these authors found that 3 to 4 per cent  $\text{CO}_2$  caused no deterioration in either manual or arithmetical skill and some

subjects showed no deterioration even with 6 per cent  $\text{CO}_2$ . They concluded that the  $\text{CO}_2$  should be kept below 0.3 per cent in air at 10 atm pressure.

Psychological effects and changes in emotional states were also experienced when breathing  $\text{CO}_2$  while under increased air pressure and perseveration was noted in some subjects. One of the more notable psychological symptoms was that of euphoria and elation but states of depression, fear, and loss of memory were also reported. Such reactions correspond surprisingly well with the psychological effects which have been described (Behnke, Thomson and Motley, 1935) as typical of the reactions to compressed air and which have been attributed to "nitrogen narcosis."

A consideration of the evidence, then, reveals not only a high probability that  $\text{CO}_2$  is an important contributor to, if not the chief cause of, those reactions which have been attributed to a narcotic action of nitrogen, but also, because the effects of  $\text{CO}_2$  are so markedly enhanced by a compression of the respiratory medium, that the results of experiments with increased  $\text{CO}_2$  tension at atmospheric pressure do not safely exclude  $\text{CO}_2$  as an etiological factor of those reactions.

5 *Physical factors in relation to  $\text{CO}_2$  effects* Although Case and Haldane (1941b) observed that at increased air pressure the resistance to breathing through canisters was at times "intolerable" they concluded that the physical properties of the gases in the gas phase could not account for the subjective effects induced by compressed air. This conclusion was supported by their finding that in shifting from various  $\text{He-O}_2$  and  $\text{H}_2\text{-O}_2$  mixtures to air and to  $\text{N}_2\text{-O}_2$  mixtures at the same high pressure, the onset of the subjective effects was not immediate but rather was delayed for a few minutes. The possible influence of physical factors, however, deserves a re-evaluation, not so much because of any direct physical action which the diluent gases for  $\text{O}_2$  may have on the organism, but rather because of the influence which the physical properties of those gases may exert indirectly by their effect on  $\text{CO}_2$  as an immediate causative agent of compressed air intoxication.

The short delay (1 to 3 min) in the onset of the subjective response to compressed air stressed by Case and Haldane (1941b), the immediate onset emphasized by Behnke et al (1935), and the observations of Shilling and Willgrube (1937) leave no doubt but that the subjective effects of compressed air are rapid in their onset. This rapidity of onset has been stressed as one of the characteristics of "nitrogen narcosis," yet this rapidity of onset serves as well or even better as evidence in support of the interpretation that  $\text{CO}_2$  is the etiological agent in compressed air intoxication.

A consideration of this characteristic of the reaction reveals that the rate of compression assumes particular significance both in the rapidity of symptom onset and the severity of those symptoms, even though any possible effects of pressure *per se* on the tissues or compressional effects on gas pockets in the body, be excluded. In very rapid compression the movement of the compressional air into the lung during the process of pressure equalization will completely prevent the exhalation of any alveolar air in spite of the respiratory movements—a simple experimental observation which the reviewer has made on numerous occasions.

Under such conditions the alveolar  $\text{CO}_2$  which would normally find its way out into the alveolar ducts and terminal bronchioles, will be pushed back into the alveoli so that even the dead space may not be available for diluting the alveolar  $\text{CO}_2$ , the tension of which will thereby be increased. In the meantime the flow of  $\text{CO}_2$  into the alveoli from the venous blood will have continued. The resultant sharp rise of alveolar  $\text{CO}_2$  tension will have reached a maximum just at the end of the compressional period. But before that time the  $\text{CO}_2$  tension of the tissues will have been appreciably elevated due to the damming up of the  $\text{CO}_2$  in the lungs and thus, associated with a concomitant increase of  $\text{O}_2$  tension in the compressional air should be rapidly reflected in both subjective and objective changes in the subject. An early manifestation of symptoms is exactly what might be expected. This sequence of events provides a very feasible explanation, then, for the dizziness and other subjective reactions which as Shilling and Willgrube (1937) point out occur in divers when rapidly compressed.

The possible influence of the compressional inflow on alveolar  $\text{CO}_2$  tension is brought out by the following simple calculation. If it be assumed that the volume of the residual and supplemental air in a man is 2500 cc and that it contains 5.5 per cent  $\text{CO}_2$ , there will be 137.5 cc of  $\text{CO}_2$  left in the lungs at the end of a normal expiration. If further, the  $\text{CO}_2$  in the expired air be taken as 4 per cent, the tidal air as 500 cc and the respiratory rate as 18 per minute, there will be expired 360 cc of  $\text{CO}_2$  each minute under normal conditions at rest, and if the organism be in a steady state, 360 cc should represent the amount of  $\text{CO}_2$  poured into the lungs from the blood each minute. Now if compression in air (0.04 per cent  $\text{CO}_2$ ) is rapidly carried out to ten atmospheres there will enter the lungs,  $9 \times 2500$  cc of air (assuming the lung volume itself is not changed) and this will add 90 cc of  $\text{CO}_2$  to that already in the lungs. If it takes 3 minutes to raise the pressure to ten atmospheres, at the end of compression, other things remaining constant, there will be a total of  $137.5 + (3 \times 360) = 1226.5$  cc of  $\text{CO}_2$  in the alveolar air. If this air in the lung were at atmospheric pressure, the  $\text{CO}_2$  would only be 4.9 per cent, but in terms of partial pressure at ten atmospheres in the lung it will be 372.85 mm Hg, whereas normally it is less than 50 mm. In other words as a result of this compressional inflow the alveolar  $\text{CO}_2$  tension will have been increased by about 700 per cent. Other things, however, are not constant, for example, as the  $\text{CO}_2$  accumulates in the alveoli the diffusion gradient is lost so that  $\text{CO}_2$  does not leave the blood at the rate of 360 cc per minute and is dammed back in the tissues, but on the other hand the diver under actual diving conditions is not in a basal state so that the  $\text{CO}_2$  production is appreciably greater than that during rest as the above calculation assumes.

There is another condition which contributes to an elevation of the alveolar  $\text{CO}_2$  tension during rapid compression not taken into account in the above sample calculation but which assumes considerable importance. The advance of the compressional inflow air into the respiratory tree pushes the alveolar air ahead of it back into the alveoli. This would mean that if the interface between the advancing compressional inflow air and the alveolar air were well defined, the  $\text{CO}_2$  tension of the gas in immediate contact with the alveolar wall at the end of a

compression to 10 atmospheres would be 10 times that which obtains under conditions of normal atmospheric pressure. In other words, if the alveolar  $\text{CO}_2$  were 5.5 per cent at atmospheric pressure, the  $\text{CO}_2$  tension of the gas in immediate contact with the alveolar wall at the end of the compression would be equivalent to that of a 55 per cent alveolar content at atmospheric pressure. It is hardly likely however that the interface between the inflow and alveolar air is discrete, but even though there be some mixing of the inflow and alveolar air during the compression such mixing cannot be immediate and the  $\text{CO}_2$  tension in contact with the alveolar wall must be elevated appreciably by this factor for at least a short time.

In discussing compressional inflow there is in addition to the rapidity of the symptom onset, another peculiar feature of compressed air intoxication which is of interest, viz., that after having attained the early maximum (within 2 or 3 min.) the symptoms do not increase in severity as the pressure is maintained but may actually decrease in severity (Shilling and Willgrube, 1937, Case and Haldane, 1941b). This feature, as Shilling and Willgrube have pointed out, casts serious doubt on the "nitrogen narcosis" hypothesis. Yet in terms of  $\text{CO}_2$  and the compressional inflow it finds ready explanation as follows. Once actual compression has stopped, the compressional inflow stops, the removal of alveolar  $\text{CO}_2$  is then resumed, tissue  $\text{CO}_2$  is consequently diminished and a subsequent decrease in severity of symptoms might then be expected.

The rapidity of compression to a given pressure determines not only the *rate* of compressional inflow and thereby the degree of blockage to the  $\text{CO}_2$  exhaled from the alveoli, but also the *length of time* over which that blockage is operative and thereby the amount of  $\text{CO}_2$  which will accumulate in the alveoli from the venous blood. The effects of both the compressional inflow and its cessation, therefore, should not be limited to only the very rapid compressions, even in the slower compressions they would appear to be significant.

Another characteristic of compressed air intoxication which has been accepted as a peculiarity of compressed air intoxication (Case and Haldane, 1941b) is the remarkably rapid disappearance of the symptoms on decompression. But this characteristic, too, may be explained in terms of  $\text{CO}_2$ . It is known that in the presence of increased  $\text{O}_2$  tension even a small increase in the  $\text{CO}_2$  tension has a much more pronounced effect than it does under normal conditions (Gesell, 1923), it follows therefore that the removal of a relatively small amount of  $\text{CO}_2$  and the concomitant lowering of the  $\text{O}_2$  tension which occurs on decompression should cause a sharp decline in severity of the symptoms. On decompression the alveolar  $\text{CO}_2$  is being removed not only by the respiratory movements but also by a decompressional outflow of alveolar air, the alveolar air and its contained  $\text{CO}_2$  is, in effect, being sucked out of the alveoli by the drop in the environmental pressure. The  $\text{CO}_2$  diffusion gradient at the lung is thereby sharply increased and this in turn should result in a more rapid removal of  $\text{CO}_2$  from the tissues.

The report that the response to compression in a  $\text{He-O}_2$  mixture was less severe than that experienced in air (Case and Haldane, 1941b) might at first appear to be valid evidence against the inclusion of compressional inflow as a contributory

factor to the occurrence of compressed air intoxication. It must be recognized, however, that a fair comparison of the influence of compressional inflow of a He-O<sub>2</sub> mixture with that of air can be made only where the rates of compression and the O<sub>2</sub> supplied to the tissue are identical. Because the experiments of Case and Haldane, just cited, were carried out for other purposes, no rigid control of the identity in compressional rates was made, furthermore, the O<sub>2</sub> content of the He-O<sub>2</sub> mixture used was 15 per cent as compared with the 21 per cent of air—a difference which of itself is of considerable importance where the effects of CO<sub>2</sub> under increased pressure are concerned. These He-O<sub>2</sub> experiments therefore provide no reason to doubt the importance of the compressional inflow.

In any case it is particularly significant that the use of a He-O<sub>2</sub> mixture did not completely eliminate the intoxicating effects of compression as it should have done if those effects were due to "nitrogen narcosis." Moreover the effects which were experienced "passed off," as would be expected according to the CO<sub>2</sub> theory, with the cessation of the compressional inflow. Further reasons why these He-O<sub>2</sub> experiments do not provide conclusive evidence of "nitrogen narcosis" are presented below in another connection.

In addition to compressional inflow and decompressional outflow, there are other physical factors deserving of consideration as possible contributors to the occurrence of compressed air intoxication, namely, diffusion resistance, gaseous density and viscosity, all of which gas properties must influence to some degree the removal of CO<sub>2</sub> from the lungs.

Snell (1896) maintained that the increased density of compressed air decreases the diffusion of CO<sub>2</sub> and for this reason advocated particularly efficient ventilation of caissons. The relative importance of the process of diffusion in the exchange of O<sub>2</sub> and CO<sub>2</sub> between alveoli and trachea under various respiratory conditions is exceedingly difficult to evaluate accurately, especially since the degree of respiratory distention is not uniform throughout the lungs and varies with changes in the type and depth of breathing. Dean and Visscher (1941) state that "it seems unlikely that diffusion is directly involved in actual mass movement of air in the lung" but this interpretation of "mass movement" of air does not rule out the probability of CO<sub>2</sub> and O<sub>2</sub> exchange by diffusion processes in the alveoli and terminal parts of the lungs. Draper and Whitehead (1944) reported, however, that in respiratory arrest induced by pentothal sodium, the diffusion alone, was sufficient to supply the O<sub>2</sub> needs if the environment were largely that of O<sub>2</sub> and that the presence of N<sub>2</sub> either in the respiratory tract or in the environment impeded this diffusion. The outward diffusion of CO<sub>2</sub> was found to be slower than that of O<sub>2</sub>.

If it be granted that the interface between the inspired air and that in the alveoli is of spike shape (Henderson et al, 1915) the gaseous exchange between the axial flow and that in immediate contact with the respiratory epithelium of the terminal bronchioles, alveolar ducts and alveoli, must be accomplished to a large extent by the relatively slow process of diffusion. DuBois (1928) accepts the view that the gaseous exchange between alveoli and bronchioles is one of diffusion. But even though one be unwilling to agree with this view without considerable reservation, there can be little doubt but that any diffusion which does

occur within the lungs will be affected when the pulmonary gas is highly compressed

The experiments of Haldane, Kellas and Kennaway (1919) show that changes in total pressure do influence diffusion resistance and gaseous exchange at the lungs, these authors found that the effectiveness of a given alveolar  $O_2$  tension was enhanced by a reduction in the total pressure. It is therefore not entirely correct to assume that a 2 per cent  $O_2$  mixture at atmospheric pressure will, when compressed to ten atmospheres, supply the tissues with the same amount of  $O_2$  as that provided by a 20 per cent  $O_2$  mixture used at atmospheric pressure, this is a point of some pertinence to the making of respiratory gas mixtures for use at high pressures

If an alteration in diffusion resistance affects the  $O_2$  supply it must also affect the removal of  $CO_2$ , and any interference with  $CO_2$  removal will contribute to the induction of compressed air intoxication. Since very small amounts of  $CO_2$  acquire a peculiarly high potency in compressed air or increased  $O_2$  tensions, even those changes in diffusion resistance which under ordinary conditions would be inconsequential, cannot be dismissed as insignificant in compressed air. Although argon is an inert gas and has been considered insignificant biologically, at least when present in such minute quantities as found in normal air, its physical properties (an atomic weight almost three times that of nitrogen) are such as would make it particularly effective in augmenting diffusion resistance were it present in any appreciable amount. In highly compressed air, argon (at 10 atm it would be 9.4 per cent of 1 atm) might very well increase the diffusion resistance and so contribute, at least in small measure, to the interference with  $CO_2$  removal.

The experiments of Behnke and Yarbrough (1939) in which argon was used as a diluent for  $O_2$  are particularly significant because they point to the importance of such physical properties as diffusion, and "respiratory resistance" in exposures to compressed gas mixtures. A comparison of the subjective and neuromuscular effects induced in experienced, emotionally stable, deep sea divers by their exposure to  $He-O_2$ ,  $N_2-O_2$  (air), and  $A-O_2$  mixtures at pressures up to the equivalent of a 160 foot dive, showed that the  $He-O_2$  mixture induced the least response, the response to the  $N_2-O_2$  mixture was next in order of severity and the response to the  $A-O_2$  mixture was most intense. The "greater stupefaction and neuromuscular impairment" induced by the argon mixture, as compared with that induced by the nitrogen (air), was said by the authors to be due to "the narcotic effect of argon" which "is greater than that of nitrogen at high pressures of 4 to 10 atmospheres." Now this order of effectiveness, helium-nitrogen-argon, of the gas mixtures in the induction of symptoms of intoxication, is exactly what one would expect on the basis of the physical properties of these gases and their influence on diffusion resistance and  $CO_2$  removal. In any case the probability that the "narcotic action" of argon, nitrogen and helium may have been due to alterations in  $CO_2$  removal have not been adequately ruled out.

The density and viscosity of a gas determines to a very large extent its facility and type of flow. The exact contribution of these properties to the involvement of  $CO_2$  in the intoxication by compressed air or other gas mixtures under pressure,

aside from their influence on diffusion, is problematic, especially since viscosity of a gas may be diminished at increased pressure. In the normal lung at atmospheric pressure these properties would appear to be rather unimportant in pulmonary ventilation (Dean and Visscher, 1941). But with compression, especially where high pressures are attained, the density is distinctly altered and to such degree that a dismissal of this factor as entirely inconsequential is hardly justified without more factual evidence. Certainly if there is a flow of gas in the more terminal portions of the lung, the diameter of the smaller tubes (0.19 mm, Miller, 1937) and the possibility of their active constriction (Macklin, 1929) would enhance the importance of any change in the flow characteristics of the respired gas. Bayliss and Robertson (1939) localize the "viscance" of respired gas in the terminal bronchioles and at the entrance to the alveoli. This is supported by the anatomical findings of Baltusberger (1921) and Miller (1937) who described rings of muscular tissue of sphincters between the bronchioles and the alveoli in human lungs.

Behnke and Yarbrough (1939) were unable to detect any difference in resistance to gas flow of  $\text{He-O}_2$ ,  $\text{N}_2\text{-O}_2$  (air), or  $\text{A-O}_2$  mixtures at atmospheric pressure, but elevation of the pressure progressively increased the resistance to flow so that at 4 atmospheres it was more than 100 per cent above the value for 1 atmosphere. This increase in resistance with increase in pressure is especially noteworthy because in actual respiration tests no difference in "respiratory resistance" could be felt by the subjects breathing these same gas mixtures either at atmospheric pressure or at a pressure of 4 atmospheres. At a pressure of 10 atmospheres, however, the subjects "were able to breathe argon-oxygen for a few minutes only because of the increased resistance to breathing and the narcotic action of argon." The report of Case and Haldane (1941b) on the increased resistance to breathing in highly compressed air also points to the practical importance of the viscosity and increased density of respired gas under conditions of compression.

If diffusion resistance and the resistance to mass movement because of increased gas density are greater in compressed air than in air at normal pressure they should, other things being constant, tend to maintain a supranormal alveolar  $\text{CO}_2$  tension. The data of Hill and Greenwood (1906) and of Haldane and Priestley (1935) are of interest in this connection, these authors concluded that exposure to air at 75 pounds' pressure did not alter the pulmonary  $\text{CO}_2$  and although Haldane and Priestley interpret the data as evidence of a constancy of alveolar  $\text{CO}_2$  tension and the regulation of breathing "so as to keep the partial pressure of  $\text{CO}_2$  steady," an examination of the Hill and Greenwood data as used by Haldane and Priestley (table 2) reveals two features relevant to the question of the effect of increased pressure on  $\text{CO}_2$  removal—especially if the breathing were regulated as claimed.

The data show first, that with few exceptions the alveolar  $\text{CO}_2$  tension even with a relatively small increase in air pressure was higher than that which could be accounted for simply by the  $\text{CO}_2$  in the ambient air. Furthermore, this elevation of alveolar  $\text{CO}_2$  tension could not have been caused by a rapid compres-



sional inflow because the rate of compression was very slow. Granting there is no increase in metabolism under increased air pressure, this elevation of alveolar  $\text{CO}_2$  indicates a retention of  $\text{CO}_2$ . Under such conditions a new set of  $\text{CO}_2$  diffusion gradients would be built up, the organism could continue to function at a higher  $\text{CO}_2$  level and the subjective symptoms of compressed air viewed as  $\text{CO}_2$  effects need not then become progressively more severe as the air pressure is maintained. Furthermore as Case and Haldane (1941b) have observed, high percentage of  $\text{CO}_2$  at atmospheric pressure do not seem to have any cumulative action over prolonged periods of administration.

The second, and perhaps equally significant feature revealed by the data tabulated below, is the fact that the elevated alveolar  $\text{CO}_2$  tension did not drop as the pressure was lowered, as it should have done if it simply represented the  $\text{CO}_2$  of the ambient air. Even on return to 760 mm Hg the alveolar  $\text{CO}_2$  tension was still above the pre-compression value. This lag in the return to normal alveolar  $\text{CO}_2$  tension perhaps is most logically explained as due to a lag in the post

TABLE 2

ATMOSPHERIC PRESSURE	ALVEOLAR $\text{CO}_2$ -PERCENTAGE		ALVEOLAR $\text{CO}_2$ PRESSURE	
	Hill	Greenwood	Hill	Greenwood
mm Hg			mm Hg	mm Hg
760	4.7	5.3	33.5	37.8
4,640	0.75	0.9	34.4	41.3
3,860	0.95	1.0	36.2	38.1
3,090	1.2	1.3	36.5	39.5
2,310	1.8	1.8	40.7	40.7
1,540	2.5	2.7	37.5	40.5
760	5.0	5.4	35.6	38.5

decompression removal of the  $\text{CO}_2$  which had accumulated in the tissues during the exposure to increased pressure.

Reasons why the diminished severity of the subjective and neuromuscular reactions to compression by the use of the  $\text{He-O}_2$  mixture does not constitute valid evidence that nitrogen acts as a narcotic have been presented above. To these there may now be added several others which involve the physical factors under discussion.

The results of substituting helium for nitrogen in a compression mixture cannot be safely considered simply as due to the absence of a direct or narcotic action of nitrogen unless such substitution introduces no new physical conditions which might at the same time alter the removal of  $\text{CO}_2$ . But obviously enough, the substitution of helium for nitrogen in the compression mixture does alter the physical factors of resistance to gas flow (Behnke and Yarbrough, 1939), of gas density, and of diffusion, all of which may influence  $\text{CO}_2$  removal. The properties of helium are such as to facilitate the removal of  $\text{CO}_2$  by diffusion and its use should therefore diminish the  $\text{CO}_2$  effects on the organism. Dean and Visscher (1941) suggest that the faster rate of  $\text{CO}_2$  diffusion in helium-oxygen mixture

than in air may be one argument for the use of helium therapeutically. The finding that the use of helium as a diluent for  $O_2$  diminishes the subjective and neuromuscular effects of compression is, then, in accord with what one might expect if  $CO_2$  were the causative agent and such finding therefore fails to provide substantiation for the "nitrogen narcosis" hypothesis.

The fact that while the substitution of helium for nitrogen diminishes the intoxicating effects it does not eliminate them (Case and Haldane, 1941) deserves particular emphasis in this connection. The report of Behnke and Yarbrough (1938) provides additional evidence of the failure of helium to eliminate the compressional effects of neuromuscular mechanisms. A condensed tabulation of the data derived by these investigators from experiments in which a skilled typist was subjected to compressed air and to compressed helium-oxygen mixtures is presented below in table 3. This shows that while the typing speed was reduced by 17.7 per cent when breathing the helium-oxygen mixture at a pressure equivalent to that of a 250 foot depth, it was reduced only by 5 per cent when breathing air at the same pressure. These results are interestingly interpreted by the authors who say, "Impaired judgment, a characteristic effect of high air pressure, is brought out by the fact that the typist realizing that he was making errors

TABLE 3

PRESSURE COND. RESPIRATORY MEDIUM	SURFACE		200 FEET DEPTH		250 FEET DEPTH	
	Air	He- $O_2$	Air	He- $O_2$	Air	He- $O_2$
Words per min.	63.2	69.0	53.0	63.0	60.0	55.8
Errors per stroke	0.0032	0.0062	0.0185	0.005	0.011	0.0067

while breathing helium slowed down his rate of copy." On the other hand, the fact that raising the pressure of the He- $O_2$  mixture to the pressure equivalent of a water depth of 250 feet distinctly slowed the rate of typing, indicates that He- $O_2$  at increased pressure does alter neuromuscular processes and subjective reactions. The failure of the substitution of helium for nitrogen to eliminate the intoxicating effects of compression constitutes further evidence for questioning the "nitrogen narcosis" hypothesis in compressed air intoxication.

If the intoxication which has been ascribed to "nitrogen narcosis" occurs even in the absence of nitrogen, some other explanation is obviously called for, in the case of the intoxication induced by the argon-oxygen mixture it was concluded that argon must have been the responsible agent and that therefore it also must possess narcotic properties (Behnke and Yarbrough, 1939), on similar grounds narcotic properties must also be delegated to helium. But the intoxicating influence of these gas mixtures may, as already pointed out, be more logically explained on the basis of an alteration in removal of  $CO_2$ . In any case the data at hand do not appear to warrant the conclusion that the intoxicating effects experienced when compressed in He- $O_2$ , N- $O_2$ , or A- $O_2$  mixtures are caused by a direct narcotic action of helium, nitrogen and argon on the organism.

There are several more points relating to the use of He- $O_2$  mixtures which

would appear to be worthy of brief mention. These concern the effects of shifting from one gas mixture to another while the pressure is maintained at a constant high level. In shifting from a He-O<sub>2</sub> mixture at high pressure to air at the same pressure, there would of course be no compressional inflow of gas into the lungs. One would expect therefore that the symptom onset of compressed air intoxication would be somewhat more delayed than in those cases where the increased air pressure had been attained by rapid compression. Although no definite experimental comparison of the time of symptom onset seems to have been carried out, Case and Haldane (1941b) report that on shifting from He-O<sub>2</sub> to air it takes several minutes for the subjective symptoms to develop. This delay might be interpreted in terms of the "nitrogen narcosis" hypothesis as the time lapse necessary for the narcotic action of nitrogen to become effective, but this delay is again well explained on the CO<sub>2</sub> theory, for in the absence of a compressional inflow the accumulation of CO<sub>2</sub> would be less rapid and the subjective effects of somewhat slower onset than in those situations where compressional inflow occurred.

But in spite of the short delay in symptom onset occasioned by the absence of compressional inflow the influence of CO<sub>2</sub> should nevertheless become rapidly manifest on shifting from He-O<sub>2</sub> to air at high pressure. The greater density of the air as compared with the He-O<sub>2</sub> mixture and the increased resistance to CO<sub>2</sub> diffusion attending such a shift would immediately begin to operate in slowing the CO<sub>2</sub> removal, or at least before the air had reached the ultimate respiratory epithelium, maximal effects could not be expected, however, until the alveoli had been washed free of He. On the other hand any direct narcotic action of N<sub>2</sub> could not take place until some time after the air had reached the alveoli and was present there in relatively high concentration. The effects of CO<sub>2</sub> retention would, therefore, be expected to be earlier in onset than those of any narcotic action of N<sub>2</sub>, but in either case a short delay such as that described by Case and Haldane would of necessity be involved.

If, as some authors have insisted, the onset of subjective symptoms of compressed air intoxication is "immediate" some other explanation than either "nitrogen narcosis" or increased CO<sub>2</sub> will have to be sought. However, if by the term "immediate" a lapse of a minute or so is meant, such immediacy of symptom onset is more readily explained on the basis of CO<sub>2</sub> retention than on that of the "nitrogen narcosis" hypothesis, especially where compressional inflow occurs.

The reports of Swindle (1937) and of End (1938) introduce another suggestion concerning the action of CO<sub>2</sub> in compressed air. End (1938) states that "agglutination of erythrocytes appears to be the primary disturbance in compressed air illness and that bubble formation may be looked upon as a serious complicating factor." Swindle has maintained that carbonic acid is probably the principal known agglutination factor normally present in the body and that this, working in conjunction with some unknown factor, promotes intravascular agglutination. On this basis End has attempted to diminish the effects of compressed air by alkalinization through the ingestion of sodium bicarbonate. To date there appears to be no further evidence in support of these contentions and it would ap-

pear unlikely that agglutination contributes to the occurrence of the subjective symptoms of compressed air intoxication.

In a problem such as this of compressed air intoxication where the variables are exceedingly difficult, if not impossible to control completely, and where the evidence indicates a very high probability of an indirect, if not direct, involvement of several etiological factors, it would appear unwise to insist that the solution lies in some single key factor. Certainly the available data do show that the presence of nitrogen constitutes an important contributory factor to the occurrence of compressed air intoxication, but an analysis of those data reveals no evidence sufficiently substantial to warrant the conclusion, either that nitrogen itself has a direct narcotic action, or that "nitrogen narcosis" is the cause of compressed air intoxication. On the other hand there is an imposing array of evidence which points to  $\text{CO}_2$  as an exceedingly important, if not the prime, etiological agent, not only of compressed air intoxication but also of argon and helium "narcosis". The whole question of "nitrogen narcosis" and  $\text{CO}_2$  influence in compressional intoxication calls, however, for further critical experimental investigation.

The present data make it appear highly improbable that either the  $\text{CO}_2$  influence, or "nitrogen narcosis" if such there be, operates alone. Increased  $\text{O}_2$  tension, especially when present in excess of that equivalent to one atmosphere, must also contribute to the response to highly compressed air, either by modifying the reaction of the organism to  $\text{CO}_2$  or perhaps by a more direct effect on the cells themselves.

Psychological factors likewise cannot be completely dismissed. The fact that so much attention in the selection of the personnel for deep diving is directed to psychological and emotional stability is, in final analysis, simply a recognition that the physico-chemical adjustment of the so-called unstable individual, when exposed to conditions of stress, is peculiarly susceptible to the superimposition of the adverse changes inherent in an exposure to abnormal environments. Such selection is a general admission that psychological factors do determine in large part an individual's reactions to pressure conditions such as those met in deep diving. Because they are psychological renders them no less real and they may frequently be more menacing, than those one likes to think of as strictly physiological and with which they are inextricably interwoven.

III EFFECTS OF OXYGEN TENSIONS IN EXCESS OF ONE ATMOSPHERE, AS INDUCED BY PURE OXYGEN, HYPEROXYGENATED OR NORMAL AIR AT HIGH PRESSURE. It is only to be expected that some of the changes induced by  $\text{O}_2$  enriched air or pure  $\text{O}_2$  at normal barometric pressure would also be seen under conditions where the organism is exposed to  $\text{O}_2$  tensions in excess of 760 mm Hg, which conditions are inclusively referred to below as those of  $\text{O}_2$  at high pressure (OHP). But there are some added and distinct effects induced by exposure to OHP, not observed at lower  $\text{O}_2$  tensions, which have a somewhat more involved etiology. For these reasons the effects of  $\text{O}_2$  at high pressures appear to warrant consideration as a special phase of a more inclusive topic, although the line of demarcation is not sharp and is admittedly an artificial one.

The first investigation of the effects of OHP was that carried out by Bert (1878) as a part of his extensive study of the reactions to breathing air and various gas mixtures at pressures both above and below that of the normal barometric reading. The experiments of Boyle (1670) and of Hoppe (1857) had clearly indicated that gas emboli would in all probability be an important etiological factor in decompression illness but it was Bert's animal experimentation which first demonstrated unequivocally that the real cause of this illness was the formation of  $N_2$  bubbles in the blood and body fluids on decompression. His experiments also showed that by breathing a gas mixture of high  $O_2$  and low  $N_2$  content this danger of bubble formation during decompression was very appreciably diminished. Thus he had anticipated by about a quarter of a century von Schrötter's suggestion (1906) that breathing OHP for five minutes to wash the tissues of dissolved nitrogen before decompression was begun, might facilitate safe return to atmospheric pressure. But of all his numerous observations, the one which seems to have impressed Bert most was the finding that OHP acted like a poison, and he concluded that OHP was toxic to every living thing.

1 *Convulsive Seizures* The most striking feature of the toxic action of OHP which Bert observed was the occurrence of convulsive seizures. These he described as similar to those induced by strychnine or phenol poisoning. After their removal from the pressure chamber, dogs were observed to be in a state of tonic rigidity. He concluded that these convulsive attacks were due to the action of the high  $O_2$  tension on the C N S—a presumption which he maintained was substantiated by his observation that chloroform inhalation arrested the attacks and that the influence of OHP on peripheral tissues was not such as could cause the attacks.

Interestingly enough, many of the seizures which he observed appeared during or just after quite rapid decompression, so that some of these attacks must have been complicated by decompression phenomena. His results must have been further complicated by his failure adequately to rule out the influence of  $CO_2$  in the respired gas, and by his use of highly compressed air. (See section on etiology below.)

The susceptibility to the convulsant action of OHP, Bert found, varied in different animals, e g, small birds were convulsed by  $O_2$  at pressures of three atmospheres or an equivalent of air at fifteen atmospheres of air pressure, but dogs were more resistant and required about four atmospheres (380 per cent of an atmosphere). If, however, the exposures were prolonged, convulsive seizures occurred even at lower pressures. Thus Bert recognized three important determinants in the precipitation of the convulsive attacks, the individual susceptibility of the animal, the intensity of  $O_2$  pressure, and the duration of the exposure. Lehmann (1884) disputed Bert's interpretation and maintained the convulsions were modified asphyxial convulsions.

Bert's early observations find confirmation in the reports of many subsequent investigators. Thompson (1889) placed pigeons and a monkey in a pressure chamber and raised the pressure to 30 pounds (gauge) by the addition of  $O_2$  (i e, an environment equivalent to 2.2 atmospheres of pure  $O_2$ ). The pigeons became

drowsy and while the monkey was at first playful it gradually became dyspneic and the respiratory rate rose from 35 to 70 per minute. Raising the pressure to 35 pounds caused some slight convulsions in the monkey, but they disappeared on decompression. There was no evacuation of urine or feces during the attacks. In other experiments Thompson placed a guinea pig, a dog, and an alligator in the chamber, the dog had a convulsion in 15 minutes at a gauge pressure of 60 pounds (4.2 atmospheres of  $O_2$ ). The pressure was then lowered to 45 pounds and the animals appeared normal. After 75 minutes of exposure at this pressure and a subsequent slow decompression to normal pressure the alligator was still unaffected, but the dog and guinea pig both died in convulsions. "This experiment, with several others," Thompson says, "shows that the higher animals are sooner affected by pressure than the lower. The monkey was asphyxiated before the pigeons, the dog before the guinea pig."

The absence of any apparent effect in the alligator on the one hand and the lethal action on the guinea pig and the dog on the other, suggests that cold blooded animals are somewhat more resistant to OHP than are warm blooded. This would seem to be borne out by the finding of Cleveland (1925) that some frogs survive  $O_2$  at pressures of 3.5 atmospheres for 65 hours, which is in accord with the reports regarding the effects of  $O_2$  at atmospheric pressure on cold blooded animals referred to in section I.

Thompson, like Bert, also described post decompressional effects. Three pigeons were subjected to  $O_2$  at a pressure of 40 pounds (gauge) for 90 minutes. Only one of these was convulsed and on removal from the chamber it remained for some time with the muscles in a curious condition of rigidity and contracture, all attempts at moving it threw it into violent convulsions. The bird lay on its back for four days with neck and legs strongly flexed. Thompson thought that these peculiar symptoms may have been due to the pressure affecting the surface of the brain unduly through an opening in the skull which had previously been made for other experimental purposes, rather than to any influence of oxygen. The other two pigeons survived and were normal.

Thompson observed further that air pressure of 8 atmospheres caused convulsions in a dog on its first exposure. The next day the same animal exposed a second time was convulsed by  $O_2$  at 5 atmospheres, 6.3 atmospheres caused exceedingly violent convulsions. It was also found that whereas after decompression from 8 atmospheres of air pressure the animal was normal, after decompression from  $O_2$  at 6.3 atmospheres the animal had tremors, inco-ordination, salivation, staring eyes, and jerky dyspneic respiration, but there were no rales in the chest. Ether was given to control the convulsions which continued for some time and the animal survived.

Smith (1899) made a somewhat more extended study of the effects of OHP and noted that mice, rats, guinea pigs, small birds and pigeons responded to OHP in a manner essentially similar to that described by Bert. The occurrence of convulsive seizures while the animals were still under pressure was, however, more commonly observed. Restlessness, dyspnea, and minor temporary muscle spasms frequently preceded the convulsive attacks. Smith reasoned that if

OHP were toxic to the C N S, it should also cause damage to those tissues, particularly the lung, through which it had to pass on its way to the C N S. His post mortem examinations of the exposed animals revealed very distinct and often extensive lung damage, thus verifying his contention. It was also found that the tension of  $O_2$  required to elicit the convulsive reaction was much higher than that required to produce the pulmonary damage and that when the lungs were thus damaged the tension required to produce convulsions was higher than when the lungs were normal.

Hill and Macleod (1903c) confirmed Smith's experimental results but their animals were somewhat more resistant to the toxic action of OHP. Smith had reported that  $O_2$  at a pressure of 1.8 atmospheres caused death in about 24 hours and that  $O_2$  at 3 atmospheres produced pulmonary inflammation in about 5 hours, whereas Hill and Macleod observed no symptomatic changes in a mouse after 6 hours of exposure to  $O_2$  at 3 atmospheres. One animal survived 9 hours' exposure to  $O_2$  at 4 atmospheres, cleaning movements, salivation, and jerky deep respiration preceded the convulsive attack which was followed by coma. Death, it was found, might occur even in the absence of convulsions, for some rats died after becoming simply dyspneic and comatose. At  $O_2$  pressures of 3 to 3.5 atmospheres no convulsions were observed, but at 4 to 5 atmospheres they commonly were seen in mice, rats, rabbits and cats. Exposures of mice and birds to  $O_2$  pressures of from 6 to 25 atmospheres quickly caused intense dyspnea and coma, but no convulsions,  $O_2$  at from 50 to 70 atmospheres instantly threw mice into convulsions resembling those of acute asphyxia and death rapidly followed.

It was also noted that animals sometimes showed marked reflex hyperexcitability after decompression and occasionally violent tetanic spasms were evoked by handling the animals—an observation which had been made previously by Thompson (1889) and Bert (1878), and more recently confirmed by Ozorio de Almeida (1934) and the reviewer (1940, unpublished data). No convulsions were observed by Hill and Macleod in compressed air at pressures as high as twelve atmospheres during compression, thus the authors thought was because under these conditions "the process of intoxication was too gradual."

Ham and Hill (1905) found that cats were convulsed in six minutes by  $O_2$  at 50 pounds' pressure and in eighty minutes by  $O_2$  at 23 pounds. There was no relationship between the size of the animal and the time of onset of convulsions. Because of the danger of  $O_2$  convulsions these authors believed that it was unsafe to use von Schrötter's method of decompressing divers in  $O_2$  pressures of 50 pounds and upwards. The authors claimed also that "any diving apparatus fitted with an oxygen cylinder, in place of air-pump and tube, is obviously too dangerous to use for many minutes at pressures over 25 pounds and should not be used for more than thirty minutes at that pressure." Some years later, however, Hill's opinion concerning the danger of  $O_2$  was changed (Twort and Hill, 1912) and he recommended the use of  $O_2$  under pressure as an aid in prevention of caisson sickness (see OHP in decompression, below).

Bean (1931) observed that pure  $O_2$  at slightly over 3 atmospheres caused convulsive attacks in dogs anesthetized with urethane. Phillips (1931) found that exposure to  $O_2$  at pressures of 45 pounds quickly leads to convulsions in animals.

In mice and rats the convulsive period was preceded by extensive washing operations which later changed into running convulsions, after a few seconds these were usually followed by a period of inactivity. When the pressure was lowered, convulsions were again observed, even in some animals which had not been convulsed while under the increased pressure. In  $O_2$  at pressures below 45 pounds animals were found to be quite free from convulsions but in prolonged exposures they seemed to become stuporous.

The observation that convulsive seizures are not infrequently precipitated during decompression from high oxygen pressure calls attention to the possibility that  $O_2$  emboli might be the responsible agent, especially since  $O_2$  bubbles have been found in the blood of animals rapidly decompressed from OHP (Hill and Macleod, 1903c, Dorello and Rowinski, 1938). Hill and Macleod (1903c) were of the opinion that those convulsions observed in their own experiments during decompression from high  $O_2$  pressure were embolic effects. Convulsions which occurred during rapid decompression from compressed air were soon followed by paralysis but those occurring in decompression from high  $O_2$  pressures continued to be excited because, as the authors suggested, the  $O_2$  set free maintained the life of the tissues.

Hill (1912) further maintained that "The convulsions which Bert details as occurring in dogs are clearly decompression results, and due to the effervescence of oxygen gas in the central nervous system. The convulsions which occur during compression are due to the high tension of the oxygen in solution in the tissue lymph. They occur by no means constantly, but only in certain individuals and under certain conditions. Often dyspnoea, coma, and paralysis come on without any marked stage of exaltation. There is one sign of excitement which is almost always present in mice, and that is rapid cleaning movements of the face. The convulsions seem never to occur when the  $O_2$  tension is below 300 per cent atmospheres, or above 600 per cent atmospheres, excepting the instantaneous convulsions which precede the death of animals exposed to enormous pressures such as +60 to 70 atmospheres. We may assume that with pressures below 300 per cent atmospheres  $O_2$  the amount of gas in solution is not sufficient to excite, that with pressure above 600 per cent atmospheres  $O_2$  the inflammation of the lungs causes the collapse of the animal."

The presence of  $O_2$  bubbles in the blood, however, does not necessarily result in convulsions. Dorello and Rowinski (1938) found that  $O_2$  bubbles which formed in the mesenteric blood vessels of guinea pigs on rapid decompression from a 20 minute exposure to  $O_2$  at 5 or 6 atmospheres caused no untoward symptoms and disappeared in from 2 to 4 minutes or less, recovery was complete. Human subjects exposed to  $O_2$  at from 3 to 4 atmospheres for 30 to 40 minutes also withstood rapid decompression without showing any subjective or objective symptoms.

There is substantial evidence that those reactions precipitated by decompression from OHP are attributable to factors other than that of  $O_2$  bubble formation. Ham and Hill (1905) in testing von Schrötter's suggestion that divers exposed to compressed air should wash out the nitrogen dissolved in their tissues by breathing pure  $O_2$  for 5 minutes before decompression, found that experimental animals were intensely convulsed by rapid decompression following a five minute ex-



posure to  $O_2$  at a pressure of 20 atmospheres, yet an examination of the blood showed surprisingly few bubbles. Further evidence that bubble formation is not the sole, or even the chief cause of these decompression convulsions is the difficulty which has frequently been experienced in safely returning dogs to atmospheric pressure even where  $O_2$  bubble formation has been eliminated by very slow decompression after exposures to OHP for several hours (Bean and Rottschäfer, 1938). Similarly the reviewer has found that convulsive seizures commonly occurred in unanesthetized rats during very slow decompression from relatively short exposures to OHP, these again cannot be explained as due to bubble formation. Some rats are peculiarly susceptible to audiogenic convulsions so that the noise of escaping gas during decompression may contribute to the precipitation of the decompressional convulsions.

The responses of isolated smooth muscle (Bean and Bohr, 1940) indicate that decompression from OHP does not immediately initiate recovery, indeed decompression appears at times actually to cause a temporary exacerbation of the effects induced by the OHP. It may be that this decompressional effect, which cannot be explained either by bubble formation or lung damage, is a response caused by a shift in function of enzyme mechanisms within the tissues. The situation would then be essentially similar to that proposed above as a possible explanation for the failure of animals adequately to readjust themselves to normal atmospheres after having been "acclimatized" to  $O_2$  rich atmospheres at normal pressure.

Shilling and Adams (1933) described experiments on guinea pigs, rabbits and cats in which successive  $O_2$  convulsions, characterized by extensive rigidity of the entire body, occurred at short intervals, these seizures later became continuous and ended in death. No attacks occurred in a two hour exposure to  $O_2$  at gauge pressures of 35 pounds or below, but all species developed convulsions within two hours at pressures of 55 or 60 pounds. Considerable individual variation in the susceptibility to  $O_2$  convulsions was noted. Rabbits were more susceptible than guinea pigs and rats, cats were relatively quite resistant. The lung damage induced by the  $O_2$  and the occurrence of convulsions were not causally related. These authors believed their results "constitute a warning against the uncontrolled use of pure oxygen for rapid decompression of divers and in the submarine 'lung' when escaping from great depths." Ozorio de Almeida (1934) likewise found both convulsive responses and pulmonary pathology occur in animals as a result of exposure to OHP.

Dionessow et al (1934) in accord with other observers, noted convulsions in warm blooded animals exposed to  $O_2$  at pressures up to 8 atmospheres and remarked that there were wide individual differences in susceptibility, but contrary to the reports of others (Hill and Macleod, 1903c) Dionessow et al found frogs were immune to the convulsant action of OHP. The authors maintained that the susceptibility in the higher animal forms is directly related to the greater CNS development. Iwanow et al (1934) reported that decerebration and removal of the optic thalamus prevented the convulsive seizures of  $O_2$  poisoning as did also certain types of anesthesia.

Morphine-urethane as used in routine anesthesia however does not prevent  $O_2$

convulsions, although with heavy doses of such anesthetic the severity of the attacks is diminished (Bean, 1929, 1931) Shilling and Adams (1933) reported that nembutal and veronal prevent the convulsions but not the lung damage of  $O_2$  poisoning In dogs anesthetized with sodium diethylbarbiturate, Behnke, Shaw, Shilling, Thomson and Messer (1934) observed modified convulsive seizures, characterized by violent spasms confined to the muscles of the head, neck and thorax, and accompanied by a pronounced respiratory reaction Bert (1878) found ether anaesthesia relieved  $O_2$  convulsions

Pure  $O_2$  at atmospheric pressure tends to diminish the depth of anaesthesia (Davidson, 1925), and when the  $O_2$  pressure is increased to several atmospheres the release from anaesthesia appears to be even more rapid and pronounced (Bean, 1931) Such an effect introduces the complicating possibility that reactions interpreted as convulsive seizures in anesthetized animals may in actuality represent a struggle of the animal due to lowered anaesthesia

In order to eliminate the complicating influence of OHP on chemical anaesthesia, Bean and Rottschaefer (1937, 1938), unaware of the report of Iwanow et al (1934) resorted to decerebration and decortication as a means of anaesthesia for experiments to be carried out with OHP Contrary to the observations of Iwanow et al it was found that severe convulsive attacks involving clonic and tonic features occurred in both the decorticate and decerebrate animals. The onset of such attacks was usually heralded by a distinct twitching of the external nares, lips, or some other facial muscle Alterations in respiration always preceded the convulsive seizures These respiratory changes were usually typified by a gradually developing hyperpnea which became jerky, irregular and panting in nature, later there developed a deepening and slowing with pronounced dyspnea. The expiratory effort became progressively more violent and assumed the appearance of a tonic convulsive expiration involving the greater part of the body musculature and was broken by rapid gasping inspirations The convulsive response spread also to the inspiratory phase and breathing was frequently stopped temporarily, clonic limb movements unrelated to the respiratory cycle merged into a tonic reaction and the animal attempted to assume an opisthotonic position Dilatation of the pupils, exophthalmos, excessive salivation, emesis, relaxation of sphincters with resulting defecation and urination were commonly observed during the seizures It was also shown that the functional activities of the carotid sinuses and bodies, as well as of the aortic arch and aortic body and pulmonary nervous mechanisms are not essential to the occurrence of the convulsive seizures of  $O_2$  poisoning Considerable individual variation was found in the susceptibility of decerebrate animals to the convulsant action of OHP just as there is in chemically anesthetized and non anesthetized animals

Preceding the convulsive attacks in  $O_2$  at about 5 atmospheres, unanesthetized rats appear to experience various paresthesias This is suggested by the peculiar behaviour of the animals, in addition to the twitching of facial muscles, the restlessness and face washing described by several investigators, the animals shake themselves intermittently and vigorously as though drying their fur and subject their genitalia to industrious cleansing processes Male rats which have experienced  $O_2$  convulsions almost invariably were found to have a very adherent

cast of hardened ejaculate of waxy consistency which filled the prepuce and urethra. In rats so affected by OHP as to render cleaning themselves impossible this cast unless manually removed, remained adherent for days, completely plugging the urethra. It is important, therefore, that male rats which have experienced  $O_2$  convulsions be examined and the urethral cast removed if the animal is to be used for subsequent study (Bean, 1941).

Ozorio de Almeida (1934) reported that the susceptibility to the toxic action of OHP was increased by successive exposures, this sensitization, it was claimed, applied not only to the convulsive response but also to the pathology induced in the lung. This sensitization was observed even in animals exposed for periods too short to induce any obvious toxic symptoms and those animals which on removal from the OHP appeared to be entirely normal were convulsed in a much shorter time when exposed again after a 24 hour interval, than were those not previously exposed. The increased sensitivity to OHP persisted for three or four days after an exposure, then gradually disappeared. This persistency of an increased sensitivity indicates that the animals do not completely or immediately recover on decompression. Further evidence that recovery is not complete even several days after decompression is the retention of a hyperexcitability and the change from a docile to a combative attitude (Ozorio de Almeida, 1934, Bean and Siegfried, 1943). Young animals were found by Ozorio de Almeida to be more resistant to the convulsant effect of OHP than adults, and starved animals withstood the OHP better than the well-fed. The observation that young animals are more resistant to the toxic action of OHP than old finds confirmation in the report of Prikladowicz (1936).

Hederer and Andre (1940) summarize the progressive responses which they observed in a rabbit exposed to  $O_2$  at a pressure of 8 atmospheres absolute as follows

DURATION OF EXPOSURE	OUTSTANDING SYMPTOMS
<i>minutes</i>	
1-5	Erection of ears with dilatation and redness of the blood vessels
5-10	Spasm of superciliary, orbicular and masseter muscles, trembling of the lips, protrusion of the eyeballs
10-15	Agitation and restlessness, spasmodic trembling of the limbs and trunk, partial or general rigidity, emission of urine
15-18	<i>Convulsive epileptiform crisis</i> with violent torsion of the trunk, somersaults, movements of the distal portion of the feet, revulsion of the eyeballs, hyper-extension of the head
18-22	Four more crises resembling combined general convulsions to the phase of tetanic rigidity
22-28	Succession of three severe crises as of the preceding type with muscular jerks localized (lips, masseters, limbs) <i>in muscle groups commonly employed</i>
29-32	Animal "affalé", motionless, slowed respiration (25-30) some clonic muscular shaking
32-36	Coma with superficial respiration and arrhythmia, death

The lethal and the convulsive threshold for animals in terms of duration of exposure and  $O_2$  pressure and that which was considered as the threshold for safety in man have been graphically represented by Hederer and Andre (1940) and are redrawn below

Hederer and Andre maintain that the acute intoxication by  $O_2$  follows the general law of mass action, that this acute intoxication which they designate as the "Paul Bert Effect" is reversible, but that the "Lorraine Smith Effect" (lung pathology) is an irreversible process. Both effects are thought to be operative in OHP and cannot easily be separated by the use of certain degrees of pressure. These authors, like Ozorio de Almeida (1934) and Dionessow et al (1934), found that animals which had suffered one exposure to OHP were more susceptible to the toxic action in subsequent exposures, a result which again implies there are

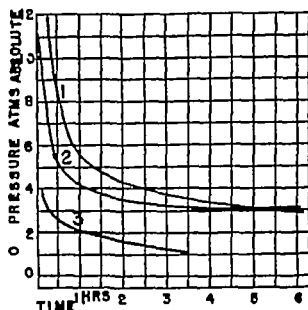


Fig 1 Curve 1 represents the threshold for lethal intoxication, curve 2 threshold for convulsive intoxication, curve 3 the safe threshold for man in diving and salvage apparatus (Redrawn after Hederer and Andre, Acad Med 123 294 1940)

residual effects of exposure to OHP and that recovery is not complete on decompression

Their results also confirm the finding of Ozorio de Almeida (1934), as do those of Campbell (1937a), that starvation plays a protective rôle against the occurrence of  $O_2$  convulsions. Carbon dioxide, added to the OHP in small amounts, was found precipitates convulsive seizures, thus emphasizing the importance of  $CO_2$  in  $O_2$  poisoning. Strychnine, it was found, accentuates the  $O_2$  poisoning but barbiturates exercise some antagonistic action, retarding, diminishing, and even stopping the convulsions.

Williams and Beecher (1944) describe various stages in the development of poisoning by OHP in *Drosophila* analogous to the motor disturbances and convulsions induced in mammals by OHP. An initial period of excitation is followed by loss of both reflex wing movement and initiation of flight, stiffening of the legs ensues and there is a loss of spontaneous movement and reflex excitability. These observations are somewhat at variance with the report of Bert that while

beetles and similar forms of life are killed by OHP they do not show any excitation or reaction analogous to convulsions

The observation by these investigators that  $\text{CO}_2$  enhances the toxic action of OHP on those forms of life devoid of hemoglobin is particularly noteworthy and is in accord with a similar enhancement which occurs in higher forms (Gesell, 1923, Hill, 1933, Shaw et al, 1934, Hederer and Andre, 1940, and others) Twenty-four hours after a 1 hour exposure to  $\text{O}_2$  at a pressure of 5 atmospheres, recovery, judging from wing beat frequencies, was apparently complete but there were residual effects following exposures of 2 hours' duration The inability of *Drosophila* to fly in air compressed to 10 atmospheres as observed by Case and Haldane (1941) may have been due in part then to the effects of OHP in addition to the increased density of the air which those authors implied may have been the cause

Valenzuela (1887) appears to be the first to have subjected man to  $\text{O}_2$  at pressures slightly greater than one atmosphere for therapeutic purposes, and Boycott, Damant and Haldane (1908) breathed  $\text{O}_2$  at a pressure of 1.7 atmospheres for a few minutes without experiencing any untoward reactions But Bornstein (Bornstein and Stroink, 1912) is to be credited with being the first investigator willing to risk exposing himself to OHP to a point of eliciting subjective and neuro-muscular responses He experienced muscular cramps after a 50 minute exposure in  $\text{O}_2$  at a pressure of 3 atmospheres The observations of Bornstein and Stroink concerning the convulsive responses and pathologic lung changes induced in dogs and rats by OHP, conform with those described by the earlier investigators

Thompson (1935) reported that in 1933 two British naval officers using themselves as experimental subjects found that breathing  $\text{O}_2$  at a pressure of 45 pounds caused symptoms of convulsions in 16 and 13 minutes In one, violent twitching of the face occurred but this was relieved by shifting to breathing air, in another there was twitching of the lips but in spite of shifting to air this passed into clonic convulsions and a loss of consciousness

Behnke, Johnson, Poppen and Motley (1935) found that one of their human subjects after breathing  $\text{O}_2$  at four atmospheres for 43 minutes experienced a transient syncope associated with blanching of the face, sweating of the hands, absence of radial pulse and a drop in blood pressure Inhalation of air brought about what appeared to be an immediate and complete recovery Another subject in a similar exposure felt well for 43 minutes, no significant changes occurred in the pulse rate, the blood pressure, the respiratory rate or minute volume, but during the 44th minute a temporary twitching of the left eyebrow was observed The subject then uttered a short cry and developed a convulsion characterized in the beginning by violent tonic movements, and then by clonic contractions of the muscles of the head, trunk and extremities Cyanosis did not occur and sphincter control was maintained With subsidence of the convulsive movements the subject was in a stuporous condition for about 13 minutes During this period the face turned an ashen gray color, beads of perspiration appeared on the forehead and breathing became stertorous When he regained consciousness

20 minutes after the onset of the convulsion, the subject thought he had fallen asleep. Recovery was said to have been complete.

In another series of experiments Behnke, Forbes and Motley (1936) found that during the first three hours of exposure of a man to  $O_2$  at a pressure of 3 atmospheres, there was a moderate facial pallor, dilatation of the pupils, impairment of visual acuity, and a rise in diastolic blood pressure, but no "distressing" symptoms. A period of threatened attack came on abruptly during the fourth hour and was characterized by dizziness, nausea, and a feeling of impending collapse, an increase in pulse rate, a rise in both systolic and diastolic blood pressure of 15 to 20 mm Hg, rapid contraction of the visual field to a  $10^\circ$  circle, and failure in visual acuity for form and color. Left temporal hemianopsia and unequal dilatation of pupils was observed in one subject. Consciousness was retained, but the subjects looked dazed and partially stupefied. Recovery was gradual and took place within about 20 to 60 minutes after removal from OHP. The persistence of effects for such a length of time after decompression illustrates further that the effects of exposure to OHP are not immediately reversible on decompression, as has been claimed by Shaw, Behnke and Messer (1934).

TABLE 4

DEPTH OF $H_2O$	MINUTES	DEPTH OF $H_2O$	MINUTES
100	60	200	6
120	20	250	4
150	8	300	3

The British Admiralty Committee observed that men sitting in a chamber of  $O_2$  at a pressure of 3 atmospheres showed no abnormal symptoms, but that in diving trials where the men were at work,  $O_2$  pressures of even less than 2 atmospheres caused abnormal behavior and loss of memory (Haldane and Priestley, 1935). These effects the authors say "were strongly suggestive of some influence on the brain similar to that arising from low oxygen." The more pronounced response in actual diving trials as compared with those observed in the compression chamber tests, finds confirmation in observations of Behnke and Yarbrough (1938) who, as has been mentioned above in connection with "nitrogen narcosis," found that the response to compressed air in actual diving was much more severe than that elicited by the same air pressure in an experimental compression chamber. Jenkinson (1940) agreeing with the data of Damant states that it is unlikely that convulsions will appear at various pressure in less than the time presented in table 4. The only premonition was said to be a twitching of face muscles and paresthesia in the extremities.

Haldane (1941) reported that in  $O_2$  at 7 atmospheres convulsions came on with little warning, but that there was a slight feeling of anxiety. The clonic seizures were very violent and caused a back injury which was painful for more than a year. These seizures lasted for about two minutes and were followed by flaccidity. That author says "I wake up into a state of extreme terror in which I

may make futile attempts to escape from the steel chamber, whereas, like others, I am quite calm on recovery from carbon dioxide nitrogen narcosis. At 7 atmospheres five minutes' exposure is about the limit tolerated. It is obvious that convulsions of this sort would be fatal if they occurred while a man was wearing an escape apparatus under water." An inadvertent exposure of the same subject (J.B.S.H.) to  $O_2$  at 10 atmospheres also resulted in a severe convulsion (Case and Haldane, 1941b). The observation of making futile attempts to escape from the chamber is of particular interest for it has been reported that some divers at great depth have been so affected as to attempt to unscrew the face plate of their diving helmet—an aberration which some would attribute to nitrogen effects.

**2 Breathing** Although Bert claimed that compressed air caused a change in position of the diaphragm and an increased thoracic capacity because of mechanical factors, he apparently considered that such changes were not of any determining significance in the response of animals to OHP. He recognized, however, that changes in breathing constituted a prominent feature of the convulsive attacks induced by  $O_2$  at pressures of about 3 atmospheres. During these attacks the breathing was observed to be very deep and precipitous and in successive attacks it became slower and sometimes completely stopped. On the other hand, he had also observed that sometimes the respiratory rate was as high as 50 to 70 per minute, especially in birds. Lehmann (1884) maintained that the response of mice and birds to OHP was essentially respiratory, the dyspnea, characterized by slowed deepened breathing became convulsive and was asphyxial in type.

Thompson (1889) reported that although breathing in  $O_2$  at atmospheric pressure was not materially different from that in air, in  $O_2$  at a pressure of 2 atmospheres, respiration became jerky and dyspneic. The frequency of breathing in animals with complete collapse of one lung was found to be reduced both by compressed air and compressed  $O_2$  but he thought that under such conditions the mechanical effects of the increased pressure added greatly to the distress of the animal.

Smith (1899) observed in his experimental animals exposed to OHP that embarrassment of respiration and dyspnea set in some time before death. Hill and Macleod (1903c) considered "gaping" and jerky, deep respiration as symptoms which precede the convulsions of vertebrates exposed to  $O_2$  at pressures of from 4 to 5 atmospheres and that dyspnea precedes the coma induced by exposure to  $O_2$  at pressures of from 6 to 25 atmospheres. Lehmann (1884) and Hill (1906) stated that OHP kills as if by asphyxia. Dautrebande and Haldane (1921) reported that breathing  $O_2$ , particularly at increased barometric pressure, increases breathing. Gesell (1923) observed dyspnea in rats exposed to OHP, and finding also that the respiratory response to  $CO_2$  was more pronounced under such conditions than in air at normal pressure, suggested that the effects of OHP on breathing warranted closer study. That such a study was in order was indicated also by the limited data and the fact that practically all of them had been derived simply from observations made through windows in the pressure chamber walls. The problem obviously called for newer methods of investigation.

It has been argued by some that to obtain reliable data the pressure chamber in such investigations should be such as to admit the operator and observer himself, that argument is, of course, quite valid for investigations on man, especially when subjective reactions are concerned. On the other hand, the presence of the observer within the chamber introduces a very distinct disadvantage and in some cases a new source of error, the importance of which has not always been recognized. Where the observer is exposed to the increased pressure, whether it be air or  $O_2$ , he too becomes an experimental animal and the data obtained under such conditions may completely lose their objectivity or be seriously distorted. This point has been emphasized (Bean and Rottschaefer, 1938) and finds some of its best support in the observations of those investigators (Behnke et al., 1934) who have advocated the exposure of the observer to the increased pressure along with the experimental animal. For example, it has been found (Behnke, Thomson and Motley, 1935) in an observer exposed to increased air pressure of from 3 to 4 atmospheres that "the palpation of the pulse of another worker was accomplished only with extreme difficulty" and that there was "partial stupefaction." All of the entire group of laboratory workers so exposed were reported to be affected emotionally, mentally and neuromuscularly, to such a degree that normal conduct could be maintained only with a great degree of self-control, that the mental activity was slowed and the responses to visual, auditory, olfactory and tactile reception were delayed, errors were frequently made in arithmetical calculations and in the recording of data.

The findings of Case and Haldane (1941b) offer further evidence that when possible the observer should not be subjected to the same conditions of pressure as the animal to be studied. These authors state that in air at increased pressure "It is quite imperative that no great trust should be placed in human intelligence." One of their subjects who was a "responsible scientist at atmospheric pressure" was detected "cheating" in a manual dexterity test while under pressure. The effects described by Case and Haldane have been attributed to increased nitrogen tensions, but increased tension of  $O_2$  may also deprive the investigator of his objectivity, a man, who, after breathing OHP for 43 minutes "suddenly experienced a transient syncope associated with a blanching of the face, sweating of the hands, and absence of the radial pulse" (Behnke et al., 1934) would seem to be in no condition to make objective observations. The report of Barcroft (1938) concerning subjective effects of  $CO_2$  is also of interest in this connection.

It would appear from the literature, then, that except where the observer's own subjective responses are under investigation, the data obtained through an operator who is himself subjected to the same pressure as the experimental animal are very probably highly coloured by his own psychological, neuromuscular and mental responses and should be accepted only with considerable reservation. It is of paramount importance, therefore, that, wherever the investigations on high  $O_2$  or air pressure permit, the regulation of the experiment and observations should be made by operators from without the compression chamber.

Many of the disadvantages encountered in the use of either the older methods



of simple visual observations or in those methods necessitating the presence of the operator within the chamber have been circumvented by the use of apparatus (Bean, 1929, 1931) which permits the continuous graphic recording of breathing and respiratory minute volume, blood pressure, heart rate, volume flow of blood, and blood acidity changes. Such apparatus also provides means of observing blood color changes, of sampling the continuously circulating blood, and of making intravenous injections while the animal is maintained at high pressure, yet the animal remains throughout, as completely under control as it would were the operator with it inside the chamber. Graphic recordings of respiration obtained by these methods showed that exposure to  $O_2$  of from 3 to 5 atmospheres caused a gradual increase in respiratory minute volume. Usually both increased rate and depth of breathing were evident, but very commonly the increased depth was the more prominent change. Not infrequently there occurred an initial slowing of breathing immediately after raising the pressure. In the shorter exposures (about one hour duration) the increased respiratory minute volume induced by the OHP was reversed by decompression to atmospheric pressure. The experiments of Shilling and Adams (1933) likewise emphasize the respiratory response to OHP. They found that in guinea pigs the respiratory rate was increased during the early minutes of exposure to increased oxygen tension, then slowed somewhat but the dyspnea just preceding the convulsions was in all cases quite marked.

In decerebrate animals exposed for more prolonged periods to OHP the convulsive seizures were usually preceded by a relatively sudden alteration in the hyperpnea induced by OHP (Bean and Rottschäfer, 1937, 1938). These alterations showed considerable individual variation, but as a rule there occurred a secondary slowing of the respiratory frequency as the exposure was continued. Each rapid gasping inspiration was immediately followed by forcible expiration, rapid in the early part of the phase, but slower as the expiratory force was mobilized from an involvement of practically the whole body musculature in a tonically spasmodic expiration. A shift from OHP to air at the same pressure very commonly was followed by a partial return toward the normal breathing. Great difficulty, however, was experienced in returning the animals to  $O_2$  at one atmosphere pressure after exposure of 3 to 6 hours in  $O_2$  at pressures of 5 atmospheres, even though the decompression was carried out well within the range of safety from bubble formation.

During decompression from prolonged exposure to OHP the respiration not uncommonly diminished, in both depth and frequency, to complete cessation and after a period of apnea, followed by a few short terminal gasps, the animal died. Recompression in  $O_2$  during the apneic period frequently caused a resumption of breathing. This difficulty in returning the animals to atmospheric pressure after prolonged exposure to OHP recalls that encountered in returning animals to normal atmospheres after their prolonged residence in hyperoxygenated air or in pure  $O_2$  at atmospheric pressure. There are, no doubt, some etiological features common to both conditions, e.g., an alteration of pulmonary membranes and perhaps incompletely or slowly reversible changes in the respiratory enzyme

mechanisms of tissue cells, but it may be worthy of note that whereas return to normal air after prolonged exposure to  $O_2$  at atmospheric pressure is attended by hyperpnea and dyspnea, the return to normal pressure after exposure to OHP is at times attended by apnea.

The respiratory response observed during the prolonged exposures to OHP was frequently so very similar to that induced by cold block application to both vagus nerves while breathing  $O_2$  at atmospheric pressure as to suggest that the OHP had effected a functional vagal block, at least in so far as respiration was concerned (Bean and Rottschäfer, 1938). These same investigators also found the hyperpnea and the convulsive respiration induced by OHP was not dependent upon either the carotid bodies and sinuses or upon the aortic bodies and mechanoreceptors in the aortic arch, although it was noted that such reflex mechanisms do contribute to the reactions to OHP elicited in the intact animal.

The respiratory response to OHP is not identical in all animals, and, like the susceptibility to the convulsive action of OHP, shows considerable individual variation. In some animals exposed to OHP the breathing after an initial hyperpnea becomes periodic (Bean, 1932). This periodicity varies in degree and type, in some instances very prolonged apneic periods (30 min) have been recorded. Accompanying this respiratory periodicity are equally pronounced rhythmic changes in volume flow of blood. Occasionally it has been noted that those few animals which failed to respond to OHP by hyperpnea have succumbed early in the exposure, this has been especially true in decerebrate animals, and suggests the importance of the compensatory function of hyperpnea in OHP. It has also been noted (Bean and Rottschäfer, 1938) that the respiratory and neuromuscular responses to OHP in animals which were decerebrated under temporary evipal anesthesia were much less pronounced than in those prepared under ether.

Behnke, Shaw, Shilling, Thomson and Messer (1934) and Shaw, Behnke and Messer (1934) have also noted the spasmodic nature of the respiratory response to OHP, as a part of, or very closely associated with, the convulsive seizures in dogs anesthetized with sodium diethyl barbiturate. These authors reported that in dogs which suffered  $O_2$  convulsions, normal breathing was resumed on shifting from OHP to normal air, and that recovery was immediate and complete. In human subjects it was observed (Behnke, Johnson, Poppen and Motley, 1935) that although breathing  $O_2$  at a pressure of 4 atmospheres might cause hyperpnea, severe  $O_2$  convulsions sometimes occurred without any preceding respiratory, circulatory or subjective changes.

3 *Metabolism* (a) *Respiratory exchange* Bert (1878) found that both the  $CO_2$  exhaled and the  $O_2$  absorbed by his experimental animals were decreased as a result of exposure to OHP, these changes were accompanied by a decrease in body temperature. The validity of Bert's results has been questioned by Krogh (1916). But Hill and Macleod (1902, 1903a, b, c) also found a diminished  $CO_2$  output and  $O_2$  absorption and a drop in body temperature in mice, rats and young rabbits exposed to  $O_2$  at pressures of one atmosphere and above, and maintained that such metabolic changes are a sign of  $O_2$  poisoning. In further agreement

with Bert these authors observed similar effects were induced by compressed air at pressures above 5 atmospheres

According to Bert air at a pressure of 10 atmospheres exerts the same effects and gives the same diminution of  $\text{CO}_2$  output and  $\text{O}_2$  absorption as  $\text{O}_2$  at a pressure of 2 atmospheres, but Hill and Macleod (1903c) found air at a pressure of 10 atmospheres to be more damaging than  $\text{O}_2$  at 2 atmospheres. They considered that the diminution of  $\text{CO}_2$  in the expired air of animals breathing OHP and compressed air at pressures up to 100 pounds "might conceivably be due to one of two causes, to some impediment to the excretion of  $\text{CO}_2$  from the blood in the lungs, in which case the  $\text{CO}_2$  content of the blood would rise, or to diminished oxidation in the tissues, in which case it would fall. Our present results," they said, "would so far tend to point to the latter as the more probable condition" (Hill and Macleod, 1903a). They later concluded that compressed air at 5 atmospheres and upwards diminished the  $\text{CO}_2$  output and increased the loss of body heat. "The lessened  $\text{CO}_2$  output is due to the high partial pressure of oxygen which arrests the oxidation processes in the tissues. A partial pressure of 100 per cent atmosphere of oxygen has this effect, and the effect increases with the partial pressure" (Hill and Macleod, 1903b).

In subsequent studies on man concerning exposure to air pressures as high as 75 pounds, Hill and Greenwood (1906) obtained data which "support the conclusion that changes in the percentage of carbon dioxide in the alveolar air depend solely upon physical conditions. No increase or decrease in the pulmonary output of  $\text{CO}_2$  occurs. Metabolism then, in so far as it can be determined by an investigation of the alveolar air, is not affected by increasing the barometric pressure. It is scarcely necessary to add that this criterion is by no means adequate to sustain the final conclusion that metabolism is, in fact, unaltered by the atmospheric conditions, so far as it goes, however, it is in favor of such an inference." They concluded, "It is probable the changes in the percentage of carbon dioxide in the alveolar air are conditioned solely by physical variations, and not by an increase or diminution in the respiratory metabolism." Hill (1906, 1912) reiterates conclusions previously stated (Hill and Macleod, 1903b) that compressed air or increased  $\text{O}_2$  pressures diminish metabolism. Bean (1929, 1931) found that the  $\text{O}_2$  absorption of anesthetized dogs was diminished by their exposure to  $\text{O}_2$  at from 3 to 5 atmospheres.

(b) *Nitrogen metabolism, blood chemistry* Bert claimed that increased  $\text{O}_2$  caused a diminution in the urea output. Hill and Macleod (1903c), questioning Bert's urea data on the grounds of insufficient preliminary determinations and because some of the data was frankly suggestive of error, carried out three test experiments on three dogs, one of which succumbed on the second day of exposure to compressed air at a pressure of from +7 to +8 atmospheres. They found no evidence of any consistent alteration in urea output, but it would appear on examination that these experiments, like those of Bert, were not entirely satisfactory and that they offer no very conclusive evidence concerning the effects of OHP on nitrogen metabolism. Shilling et al (1934) report that dogs exposed to  $\text{O}_2$  at about 45 pounds' gauge pressure showed no significant changes in urine

from normal, although in most cases it was of high specific gravity at the end of the experiments

The changes in blood chemistry reported by some authors may be a reflection of alterations in metabolism induced by OHP. Bert found that the destruction of blood glucose was diminished in animals by their exposure to OHP. Iwanow et al. (1934) reported an increase in blood sugar and dismissed the supposition that  $O_2$  convulsions were of hypoglycemic origin. Somewhat similarly, Shilling et al. (1934) reported that in animals which had had convulsive attacks in OHP, there was an increased glucose and phosphorus content of the blood but that in nonconvulsed animals there was no change. While the elevation in blood sugar and phosphorus found by these authors in the convulsed animals was considered by them to have been the result of the toxic effect of the OHP, they concluded that such elevation was not the cause of either the convulsions or the lung damage found in the animals. Determinations of non protein nitrogen, chlorides, creatinine, calcium and potassium of the blood showed either no change, or where changes did occur they were apparently not related to convulsive seizures or other symptoms of  $O_2$  poisoning. Ishikawa (1939) reported that the blood sugar was increased, but that the blood lactic acid, albumin, and the colloidal osmotic pressure of the serum was decreased in rabbits which had been exposed to compressed air at from 60 to 180 pounds' pressure. Bean and Haldé (1932) found a reversible increase in blood lactic acid of urethanized dogs exposed to  $O_2$  at pressures of 5 atmospheres and suggested this may have been due to alterations in oxidative mechanisms.

(c) *Body temperature* In considering changes in body temperature as an index of metabolic alterations induced by exposures to OHP several physical factors are worthy of attention. Hill and Macleod (1903c) reported that the increased thermal conductivity of the compressed gas contributes in large measure to the lowering of the body temperature observed in animals exposed to compressed air or to OHP. In addition to this factor of conductivity, however, there is also the possibility that the environmental temperature changes occurring as a result of rapid compression and decompression introduce significant complications, not only because of the direct effect which such environmental changes would have on body temperature but also because of the influence they would exert secondarily on metabolism and physiological processes, through the changes in body temperature.

The observation (Case and Haldane, 1941b) that the heat developed "due to adiabatic compression" was of such magnitude as to cause discomfort in human subjects is of interest in this connection. Another possible physical source of error and one which may be very significant where body temperature changes are at best not great, is the mechanical effect of pressure on some thermometers. The reviewer has observed that even with pressures as low as 50 pounds this error may amount in some cases to as much as  $1^\circ C$ .

Bert (1878) reported that exposure of animals to OHP caused a drop in body temperature as great as  $10^\circ C$  but the readings were taken after decompression. Valenzuela (1887) observed that exposure of a pneumonia patient to  $O_2$  at a pres-

sure of about 1.5 atmospheres resulted in a lowering of the temperature to 17°C. Thompson (1889) noted a decrease in the body temperature of monkeys, pigeons, guinea pigs and dogs on their return to normal atmospheric pressures after exposure to OHP. A guinea pig which died in convulsions three minutes after removal from the pressure chamber showed a drop in temperature of 14°C. The body temperatures of a dog and a guinea pig which had suffered convulsions on decompression from an exposure to O<sub>2</sub> at a pressure of 4.2 atmospheres for one hour were decreased, both animals succumbed. Thompson, however, stated that the temperature change was "in no wise associated with an increase in the O<sub>2</sub> absorbed." On the other hand the temperature of an alligator which had been exposed to the OHP along with the dog and guinea pig, but which showed no symptoms of O<sub>2</sub> poisoning either during the exposure or on decompression, increased from 51.25°F to 75°F (environmental temp had been 66°F). Cleveland (1925) reported that frogs were more resistant to OHP than warm-blooded animals. Such results suggest that this greater resistance of poikilotherms to the convulsant and toxic action of OHP is related to inherent metabolic processes as seems to be true also for the greater resistance offered by these animals to the adverse effects of increased O<sub>2</sub> tensions at atmospheric pressure. (See section I.)

No definite change in rectal temperature was observed in dogs during their exposure to O<sub>2</sub> at pressures of from 3 to 5 atmospheres at 25°C for periods of about 2 hours (Bean, 1931). Ozorio de Almeida (1934a) however found a decrease in rectal temperature of animals exposed to OHP and concluded that a decrease in organic oxidations is the essential characteristic of poisoning by OHP. Di-onessow et al (1934) also found a decreased temperature in exposures to OHP. Hederer and Andre (1940) noted a small decrease about 1°C in the body temperature of rabbits on their return to normal atmospheric conditions after exposure to OHP.

(d) *Alterations in metabolism induced by artificial means and their effect on the response to OHP.* Some further data concerning the relationship between metabolism and the effects of OHP have been obtained from various experiments in which exposure to OHP was combined with procedures which are known to, or thought to, alter metabolism. Ozorio de Almeida (1934b), in attempting to increase the destructive action of OHP on tumors through metabolic changes, used, in preliminary experiments, injections of dinitrophenol, benzole, paradichlorobenzene, quinine, nitrate and nitrite of sodium, methylene blue, prolan, and also a preparatory period of anoxemia. Starvation was found to increase the resistance of the animals to poisoning by O<sub>2</sub> at high pressure. This effect of the nutritive state of the animal on its susceptibility to O<sub>2</sub> poisoning has been confirmed by Campbell (1937a, b) and Hederer and Andre (1940).

In further attempts to alter the susceptibility of animals (80 gram rats) to O<sub>2</sub> poisoning, Campbell (1937b, 1939) administered a number of preparations, thyroxin (0.4 mgm), dinitrophenol (1.5 mgm), actetrahydro-B-naphthylamine (0.5 cc, 1 per cent), adrenaline (0.02 mgm), pituitary extract (posterior lobe, above 3.5 units), insulin (0.025 U), and eserine (0.045 mgm administered with atropine 0.075 mgm), and ergotomine (slight effect), enhanced the O<sub>2</sub> poisoning.

Glucose injections did not alter the effects of OHP, which is perhaps significant in view of the observation that a well fed animal is more susceptible to  $O_2$  poisoning than one which has been starved. Histamine, curarine chloride, acetylcholine (with eserine and atropine) were also without effect on  $O_2$  poisoning, although some increase in histamine has been found in the blood in animals poisoned by  $O_2$ . Urethane in the usual doses was found to have no effect on  $O_2$  poisoning, but dial did exert some slight protective action.

Barbiturates have also been reported (Hederer and Andre, 1940) to exert a protective action, and Behnke, Shaw, Shilling, Thomson and Messer (1934) believed the convulsive attacks were modified by barbital anesthesia—only two out of nine dogs so anesthetized had convulsive seizures. In experiments where Evipal was tried as a transient anesthesia for decerebration (Bean and Rottschaefer, 1938) the animals were less reactive to subsequent  $O_2$  exposures than were those decerebrated under ether or urethane, Evipal, it appeared, exerted some residual effect, but whether this lack of response represents a protective influence may perhaps be debated, since some animals that fail to show an increased respiratory reaction succumb early in the exposure to OHP. The protective effect of anesthetics is thought by Campbell (1939) to be due to their lowering the metabolism of the organisms. Strychnine has been found to accentuate the  $O_2$  convulsions (Hederer and Andre, 1940).

Campbell (1937a, b) interprets the fall in body temperature which has been reported by many investigators to accompany  $O_2$  poisoning as a protective reaction on the part of the animal to the increased  $O_2$  tension. He found that when the environmental temperature was raised to  $38^\circ C$  few rats survived thirty minutes' exposure to  $O_2$  at pressures of six atmospheres, whereas in an environmental temperature of  $24^\circ C$  there were few deaths. Removal of the thyroid gland was found to increase the resistance of the rats to  $O_2$  poisoning, even when the environmental temperature was raised to  $33^\circ C$ . Hypophysectomy had some but less protective action than thyroidectomy. Comparable enhancement of the toxic action of OHP by elevation of the environmental temperature was also found by Williams and Beecher (1944) in experiments on *Drosophila* at  $34.2^\circ C$ , the rate of poisoning by  $O_2$  at a pressure of 5 atmospheres was about 8 times as rapid as at a temperature of  $14.4^\circ C$ .

4 *Circulation. Blood pressure, pulse rate.* The circulatory effects of breathing OHP will depend, in large measure, upon the general response of the animal to OHP, e.g., it would be expected that the blood pressure response in an animal which had reached the convulsive stages of  $O_2$  poisoning with its violent motor responses might be quite different from that in an animal which had not attained that degree of intoxication. It may be well, therefore, to keep in mind the possibility that the circulatory changes which occur as a result of violent motor responses precipitated by OHP may completely mask some of those circulatory changes which are truly representative of the more immediate action of OHP.

Hill and Macleod (1903c) found that the blood pressure and pulse rate of an anesthetized dog were unchanged by rapid compression to, or decompression from, an  $O_2$  pressure of 3 atmospheres. Similarly the circulation in a chloralized

rabbit was not altered by exposure to  $O_2$  at a pressure of 6 atmospheres, but rapid decompression was attended by a rise in blood pressure which, the authors suggested, was due to the noise of the escaping air. This absence of any distinct change in blood pressure during compression to  $O_2$  pressures as high as 6 atmospheres has been confirmed in anesthetized and decerebrate dogs (Bean, 1929, 1931, Bean and Rottschäfer, 1938). In 60 experiments (urethane anesthesia) in which the duration of the exposure to OHP was such as to avoid pronounced convulsive seizures (about one hour), the blood pressure increased in five instances while in the others there was either no significant change or a slight decrease. These results would seem to indicate that, except for a slight tendency to rise, the blood pressure is not appreciably affected by  $O_2$  at pressures of from 3 to 6 atmospheres if the animal has not approached the convulsive stage.

In a study of the circulatory effects of compressed air on 13 human subjects (experienced divers) Hawkins, Shilling and Hansen (1932) found that exposure to compressed air (an  $O_2$  equivalent of about 1.2 atmospheres) caused the average pulse rate to rise from 68 per minute to 73 per minute, the average systolic and diastolic blood pressures increased slightly (2 mm), but the pulse pressure and circulation were not appreciably changed. Case and Haldane (1941b) reported that exposure of human subjects to compressed air ( $O_2$  equivalent of 2 atmospheres) caused an increase in heart rate, but that the changes in the systolic blood pressure were not uniform—some showed an increase while others showed a decrease.

Shaw, Behnke and Messer (1934) observed that in dogs anesthetized with sodium diethylbarbiturate,  $O_2$  at 4 atmospheres caused an immediate or delayed drop in blood pressure which persisted for as long as 3 hours, but usually this fall was rapid and terminated in the death of the animal in less than an hour from its onset. These authors maintained that "in every case in which convulsions occurred there was a preceding fall in blood pressure and conversely we may expect that a fall in blood pressure will be followed by convulsions." They concluded that a drop in blood pressure was the first sign of the toxic action of OHP. However, where artificial hyperventilation was performed during the exposure to OHP in an attempt to keep the alveolar  $CO_2$  low, the blood pressure was maintained at about normal level except for an initial drop in blood pressure attributed to "the mechanical interference with the filling of the right heart when the dog resisted the rhythmic action of the respirator."

In later work (Behnke, Johnson, Poppen and Motley, 1935) it was reported that there occurred no changes in blood pressure of four men as a result of their exposure to  $O_2$  at pressures as high as 4 atmospheres, except in two subjects. In one of these there was a drop in blood pressure, in the other there was no change in blood pressure or pulse rate for 44 minutes of exposure, at the end of which time there developed without any warning, violent convulsions from which consciousness was regained twenty minutes later. The blood pressure was apparently not followed during this seizure but it can hardly be assumed that it remained unchanged. The absence of any premonitory drop in blood pressure in this second subject emphasizes the inadequacy of the generalization that the convulsive seizure is heralded by a drop in blood pressure.

Data from a subsequent series of experiments (Behnke, Forbes and Motley, 1936) show that in the first hour of breathing  $O_2$  at 3 atmospheres—the systolic pressure in human subjects had fallen from 132 to 100 mm Hg while the diastolic pressure of 86 mm Hg had changed but little. However, after an exposure of three hours the pressure had gradually risen to 150/104. The authors state that “impending collapse was always signalized by an increase in pulse rate, rise of both systolic and diastolic pressure of 15 to 20 points.” This latter observation is in line with other experimental findings (Bean, 1931, Bean and Rottschäfer, 1938) derived from urethanized and from decerebrate dogs in which a gradual rise in blood pressure preceded, and a further rapid rise in blood pressure accompanied, the onset of convulsive seizures, similar blood pressure changes occurred after the denervation of carotid sinuses and bodies. It has been pointed out (Bean and Rottschäfer, 1938) that blood pressure changes—particularly a drop—are not a reliable index of the onset of  $O_2$  poisoning, the toxic effects of OHP are continuous and gradual in their development and one of their earliest manifestations is an alteration in breathing.

Numerous workers have reported that exposure to OHP causes a slowing of the pulse rate (Bert, 1878, Hill and Macleod, 1903c, Hill, 1912, Dautrebande and Haldane, 1921, Bean, 1929, 1931, Behnke, Johnson, Poppen and Motley, 1935, Whitehorn and Bean, 1942). This seems to justify the generalization that OHP causes a slowing of the heart but one qualification for the acceptance of this generalization would appear again to be an absence of the complicating features of convulsive seizures or collapse. A reversible slowing of the pulse was found (Bean, 1931) in urethanized dogs exposed to  $O_2$  at pressures of from 3 to 5 atmospheres for periods of 45 to 90 minutes, provided no convulsions occurred. Behnke, Johnson, Poppen and Motley (1935) report that no significant alterations in pulse rate occurred in a man exposed to 4 atmospheres of  $O_2$  for 43 minutes. In another of their subjects there was an absence of radial pulse during collapse after a similar exposure, and although Behnke, Forbes and Motley (1936) found that in a 3 hour exposure to  $O_2$  at 3 atmospheres pulse rate fell from 90 to 57 per minute, it subsequently rose and led those authors to conclude that an increase in pulse rate always signalizes “impending collapse.” An increase in pulse rate just preceding and even during convulsive seizures has been recorded in decerebrate dogs (Bean and Rottschäfer, 1938).

It has been noted (Bean and Rottschäfer, 1938) that the tachycardia which results from vagal sectioning is not prominently altered by the subsequent exposure of the animal to OHP. This was interpreted as an indication that the bradycardia which occurs so commonly in animals with intact vagi during exposure to OHP for periods of subconvulsive duration is mediated via the vagi, and that any slowing influence which might possibly be exerted by the OHP directly on the heart or through the sympathetic innervation may be completely masked by vagal sectioning.

The increase in heart rate which has been observed so frequently with the onset of  $O_2$  convulsions in animals with intact vagi and which follows the initial cardiac slowing induced by the OHP may perhaps be explained as due to a secondary chemical blockage of vagal fibres (Bean and Rottschäfer, 1938). This in-



terpretation of the late tachycardia in exposure to OHP finds support in the report of Hill and Macleod (1903c) that in the frog heart the vagus nerve endings seemed to be paralyzed by OHP

Bert (1878) noted that the hearts of animals which had succumbed to O<sub>2</sub> poisoning were still reactive to artificial stimuli but he recognized that OHP affected cardiac function, in his protocols on the frogs which had been exposed to OHP, he made the notation that the ventricular pulsations were irregular and few in number, whereas the auricles continued to beat (40 per min) Such an alteration in cardiac rhythm might, of course, have occurred as a terminal change—particularly since the frog heart is so susceptible to changes in pH (Clark, 1913, Daly and Clark, 1921), but it suggests that the OHP may have caused heart block, although no point was made of this Bert also demonstrated that isolated frog's heart suspended in the vitreous humor of a dog was not only completely arrested by exposure to O<sub>2</sub> at pressures of 10 atmospheres for 6 hours but that it lost its excitability, while the control hearts, especially the auricles, remained active and excitable He concluded that the pace setter or ganglion of the heart was arrested much more rapidly than occurred in normal air and that the muscular elements and nerve ganglia were killed by OHP

Hill and Macleod (1903c), however, found that the heart of a frog exposed to O<sub>2</sub> at pressures as high as 50 atmospheres was not rapidly poisoned and continued to beat more than an hour under such pressure Inhibition of such hearts by artificial excitation of the sino-auricular junction was readily induced, but excitation of the vagus was without effect Lehmann (1884) found that excised frog hearts did not stop beating until after 9 hours of exposure to O<sub>2</sub> at pressures of from 10 to 14 atmospheres Bean and Bohr (1938a,b) and Bohr and Bean (1939) found that O<sub>2</sub> at from 70 to 80 pounds' gauge pressure acting on excised frog ventricles caused an initial increase followed by a late decrease, in the contraction strength, a delayed slowing in frequency, and an eventual cessation of automatic contractions The pace setter was observed to be much more susceptible to the OHP than the contractile mechanism, the muscle remained excitable to artificial stimuli long after cessation of the normal beat The conductivity appeared to be initially improved, possible late effects were not apparent in these experiments In mammalian hearts it has been shown from E.K.G. records (Bean and Whitehorn, 1941, Whitehorn and Bean, 1942) that O<sub>2</sub> at pressures of about 5 atmospheres does not cause any very rapid alterations in the conductive mechanisms but the P-R interval is frequently very distinctly prolonged and some features simulating those observed during low O<sub>2</sub> administration are present in the longer exposures

*Blood vessel caliber and volume flow of blood* If, as the evidence presented in section I would indicate, O<sub>2</sub> at atmospheric pressure induces a slight vasoconstriction, it would be only reasonable to suppose that a similar but more pronounced effect might be induced by OHP The problem, however, is not quite so simple, since OHP introduces other complicating influences

The experiments of Poiseuille (1835) and of Hill and Macleod (1902) indicate that OHP administered by the use of highly compressed air causes no alteration

in caliber of peripheral vessels Bert (1878), in examining the eye ground of dogs convulsed by OHP, noted they were strongly injected, which observation suggests vasodilatation rather than constriction Dautrebande and Haldane (1921), using an alteration in alveolar  $\text{CO}_2$  as a criterion of a changed blood vessel caliber, have maintained that the breathing of OHP induces a central vasoconstriction which acts to protect the C.N.S. against the toxic effects of excess  $\text{O}_2$ . The validity of their argument may be seriously questioned, however, especially since in their experiments no attempt was made to control either pulmonary ventilation or metabolism (See section I)

A more direct method of determining the influence of OHP on the volume flow of blood through the brain, based on the experimental evidence that the flow through the carotid artery parallels the vertebral flow (Bronk and Gesell, 1927) was used in more recent investigations (Bean, 1929, 1931) In 28 experiments on urethanized dogs the volume flow changes in the carotid artery were followed continuously throughout exposures to  $\text{O}_2$  at pressures of about 5 atmospheres for periods of from 30 to 90 minutes, no significant alterations were found except in three animals in which there was a small increase in volume flow Such results would seemingly justify the conclusion that those vessels which distribute the carotid blood are not significantly constricted by breathing  $\text{O}_2$  at 5 atmospheres for the periods mentioned, and that if there is any sharply localized portion of the brain which suffers vasoconstriction, the resulting changes in blood flow to that area must be so small as to be masked by dilatation elsewhere

Further evidence that breathing OHP does not cause central vasoconstriction is found in the observations of Behnke, Forbes and Motley (1936) that the caliber of the pial arteries of a cat was not appreciably changed by the animal's exposure to  $\text{O}_2$  at a pressure of 4 atmospheres.

There are experimental data which indicate that OHP may cause central vasodilatation, rather than constriction, because of the complicating involvement of  $\text{CO}_2$ . It has long been recognized that  $\text{CO}_2$  acting directly on blood vessels may cause a local vasodilatation Moreover, there is now very convincing evidence that breathing gas mixtures containing increased  $\text{CO}_2$  at atmospheric pressure results in a very pronounced dilatation in the pial vessels of cats, and that such dilatation overshadows any slight constrictive action which increased tensions of  $\text{O}_2$  at atmospheric pressure might exert (Wolff and Lennox, 1930) Similar dilating action of  $\text{CO}_2$  on cerebral vessels was found by Forbes (1928) The results of experiments by Gibbs, Gibbs and Lennox (1935) show that cerebral blood flow (internal jugular vein) in man is increased by breathing gas mixtures high in  $\text{CO}_2$  content and that any vasoconstricting influence of  $\text{O}_2$  is masked by the greater dilating effect of  $\text{CO}_2$  Schmidt (1934) and Schmidt and Pierson (1934) reported that  $\text{CO}_2$  causes vasodilatation in hypothalamic and medullary tissues of the brain

The importance of  $\text{CO}_2$  as a causal or contributing factor in poisoning by OHP has been recognized by numerous investigators (Thompson, 1889, Gesell, 1923, Bean, 1929, 1931, Campbell, 1929, Hill, 1933, Shaw, Behnke and Messer, 1934) and the experimental data now available leave little doubt but that under OHP there

is an accumulation of  $\text{CO}_2$  in the tissues, this  $\text{CO}_2$ , in conjunction with other and possibly more direct effects of OHP might well result in a central vasodilatation.

Behnke, Forbes and Motley (1936) found that  $\text{CO}_2$  administered to a cat during its exposure to  $\text{O}_2$  at a pressure of 4 atmospheres caused an immediate and striking dilatation of the pial vessels. Inspection of their plotted data (fig 2 below) from one experiment reveals two additional features not mentioned by the authors but which may be of considerable importance. First the vessel diameter when the animal was exposed to air at 4 atmospheres' pressure was greater than when exposed to air at normal pressure, which suggests that compressed air or its increased  $\text{O}_2$  tension caused vasodilatation. Second during the first five minutes of breathing OHP the gradient of calibre decrease was temporarily sharper than that obtaining under an equal pressure of air, but following this, the vessel calibre remained constant at its precompression value until  $\text{CO}_2$  was administered some few minutes later. The sequence of changes in this second feature might be interpreted as indicative (1), that OHP causes an initial vaso-

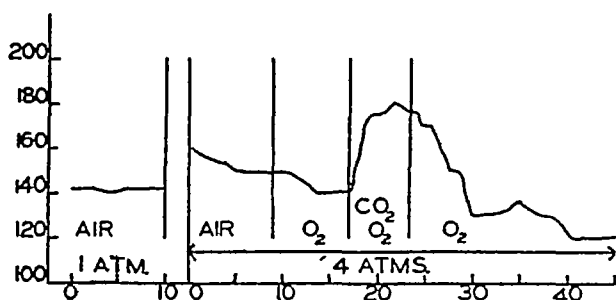


Fig 2 Changes in the diameter of a pial arteriole of a cat breathing a 2 per cent carbon dioxide (equivalent to 8 per cent carbon dioxide at 1 atmosphere) and 98 per cent oxygen mixture at 4 atmospheres' pressure. Ordinate, diameter in microns, abscissa, time in minutes (Behnke, Forbes and Motley Am J Physiol 114 436, 1935)

constriction but that this is shortly thereafter nullified by some counteracting influence which gradually comes into play, (2), that the counteracting influence may be an accumulation of endogenous  $\text{CO}_2$  in the tissues.

In view of these  $\text{CO}_2$  effects, the progressive drop of blood pressure which has been observed to precede the onset of  $\text{O}_2$  convulsions in dogs anesthetized by Dial (Shaw, Behnke and Messer, 1934) might suggest a generalized vasodilating action of accumulating  $\text{CO}_2$ . On the other hand, the maintained or gradually elevated blood pressure of urethanized dogs in the earlier stages of exposure to OHP (Bean, 1931) and the sharp rise during the convulsive seizures (Bean and Rottschäfer, 1937) would suggest that the vasodilatation which might arise from the  $\text{CO}_2$  accumulation during OHP exposure is not one of a profound generalized nature and that with the onset of strong C.N.S. effects, any peripheral vasodilatation is counteracted by vasoconstriction via the centers.

Evidence of a late peripheral vasoconstriction is found in the blanching of the skin in men about to suffer collapse from OHP (Behnke, Johnson, Poppen and Motley, 1935). A similar blanching of the ears of rats just before, during and

after convulsive seizures in OHP has been commonly observed by the reviewer. If such a peripheral vasoconstriction occurs over any very extensive area it might, of course, function to maintain or even to increase rather than decrease the blood flow to the higher centers. But in any case an evaluation of the evidence as a whole does not justify the conclusion that exposure to OHP causes predominant central vasoconstriction and decreased flow of blood to the C.N.S.

Regardless of the state of constriction or dilatation of the blood vessels in the C.N.S. the venous blood in animals exposed to OHP comes from the brain in a highly oxygenated state, as is seen from its bright red color and from the fact that such blood placed under atmospheric pressure froths as a result of the escape of the excessive  $O_2$  in solution in the plasma (Bean, 1929, 1931). Such observations clearly indicate that the organism fails to protect its C.N.S. tissues from OHP by vasoconstriction and that under such conditions the oxyhemoglobin is not normally reduced. Instead of arguing that a vasoconstrictor effect of OHP performs a protective function for the C.N.S. one might even more logically argue that greater protection might be derived from a vasodilatation which would facilitate the removal of the accumulation of  $CO_2$  and metabolites.

5 *Ferments, Micro-organisms and Enzymes* Bert reported that the potency of salivary diastase was not altered by its exposure to hyperoxygenated air at a pressure of about 15 atmospheres for 5 days. Similarly pepsin, invert ferment of yeast, myrosin and emulsin remained unaltered. In fact according to Bert, none of the soluble ferments, referred to by him as "false" or "non formed" ferments because they ordinarily retained their activity after extraction from their living source, were adversely affected by their exposure to OHP. The toxicity of scorpion venom was likewise reported to be unaltered by such treatment. Bert argued that this absence of any effect of OHP on these substances was to be expected because they were not living cellular structures. The "true" ferments, which in Bert's terminology were living cellular organisms, were however killed by OHP.

In his study on micro-organisms Bert reported that those responsible for the souring of wine were killed by hyperoxygenated air at a pressure of 10 atmospheres, putrefactive processes were likewise said to have been prevented due to the destruction or inhibition of the organisms involved. Blood was preserved for days by hyperoxygenated air at a pressure of about 14 atmospheres but hemolysis was not prevented. The coagulation of milk, too, was claimed to have been arrested by compressed air. Eggs, meat and bread were likewise preserved, although they turned acid in reaction, probably due, it was suggested, to lactic acid. Molds and yeasts were said also to be killed by hyperoxygenated air at increased pressure.

Bert reported that what he called "viruses" remained unaffected by their exposure to superoxygenated air at increased pressure. This he maintained demonstrated unequivocally that they were not living things and that their potency was not dependent upon the presence of living organisms or cells. Unfortunately the "viruses" he studied were derived from three diseases ("vaccine"—probably vaccinia, glanders, and anthrax), two of which, anthrax and glanders, are not of

virus origin, as he had erroneously assumed. Although *Bacillus anthracis* was discovered two years before LaPression Barometrique was published, the glanders bacillus was not demonstrated until 1882. Bert's own experimental finding that glanders bacillus remained virile after 6 days' exposure to hyperoxygenated air at a pressure of 20 atmospheres constitutes evidence against his own strong contention that such  $O_2$  pressures kill every living thing. Spoilage of fruit ("Blet-tissement des Fruits") was reported to have been accelerated, rather than stopped, by exposure to hyperoxygenated air at high pressure, Bert concluded therefore that that condition could not be caused by any cellular form of life and suggested that it was due to some direct oxidation.

Following this early work of Bert, various reports appeared concerning the adequacy of OHP as a sterilizing agent. Schaffer and v. Freudenreich (1891), following up some work of d'Arsenal (1891) on the effects of  $CO_2$ , failed to confirm Bert's contention that OHP killed organisms in milk. DeLavallee (1898), however, found that both aerobic and anaerobic bacteria could be killed if the cooled milk were exposed to  $CO_2$  at from 5 to 6 atmospheres' pressure for from 4 to 5 hours and then, after the  $CO_2$  had been permitted to escape, re-exposed to  $O_2$  at 5 atmospheres for 5 hours. Milk so treated, it was claimed, could be kept in a fresh condition during transportation by maintaining an  $O_2$  pressure of 2 atmospheres in the container. The practicability of the old idea of milk preservation by OHP seems to be indicated by the issuance of a U.S. Patent to K. Richter in 1941 for a method involving OHP.

Berghaus (1907) found that while 5 of the 20 different micro-organisms he studied were killed by  $O_2$  at pressures as low as 2 atmospheres, 15 remained viable after a 24 hour exposure to 35 atmospheres. He also reported that  $CO_2$  at high pressures was a much more effective lethal agent than was OHP. Cleveland (1925) demonstrated that many protozoa were readily killed by  $O_2$  pressures at 3.5 atmospheres. He also found there was a differential susceptibility between some protozoa and their hosts and suggested that this observation might be put to practical use in the defaunation of the hosts. Such procedure has been carried out with some degree of success on silkworms by Gibbs and Chen (1929). Thaysen (1934) reported that the growth of 4 micro-organisms which he investigated was retarded by  $O_2$  at pressures of 10 atmospheres, and that if the temperature were increased slightly above that for optimum growth,  $O_2$  at 10 atmospheres' pressure became lethal for these organisms. Thaysen's observation on micro-organisms is in accord then with those made on animals by Faulkner and Moore (1927) and Campbell (1937a, b) on the increased potency of  $O_2$  toxicity at increased temperature. The growth of *Pneumococcus* type I was found to be completely inhibited by exposure for 24 hours to  $O_2$  at pressures as low as 900 mm Hg and none of these organisms survived exposures to  $O_2$  of 3650 mm Hg (Bean, 1941).

These reports of experiments on micro-organisms are of interest for several reasons. They clearly indicate that the toxic action of  $O_2$  at high pressure is not limited to organisms of higher order or to animals having R.B.C.'s and a circulatory system and they provide experimental basis for seriously questioning

Bert's conclusion that no living thing can endure an  $O_2$  tension equivalent to that of 20 atmospheres of air. That this contention of Bert's is at least in need of some qualification is indicated not only by his own experiment on glands but also by the fact that the cells of swim bladders in deep sea fish are apparently immune to the injurious action of  $O_2$  even at extremely high pressures. The swim bladders of deep sea fish contain  $O_2$  in concentrations as high as 84.6 per cent (Schloesing and Richards, 1896). At depths of 4500 feet where these creatures live the pressure is in the neighborhood of 136 atmospheres which in terms of partial pressure of  $O_2$  would be about equivalent to 115 atmospheres of pure  $O_2$ .

The experiments on micro-organisms also show that in these lower forms of life as well as in higher there is a wide individual variation in susceptibility to  $O_2$  poisoning. While such variation may be due in part to structural characteristics, it suggests that differences in the respiratory enzyme systems may be responsible. The reports that  $CO_2$  and OHP in combination are more destructive to micro-organisms than OHP alone are in accord with the observation that small amounts of  $CO_2$  are particularly damaging to warm-blooded animals and points to the importance of increased  $CO_2$  on primary cellular processes under such conditions.

**6 Isolated Tissue Iris** Pupillary dilatation has been a common finding in animals exposed to OHP (Bert, 1878, Bean, 1931, Bean and Rottschaefer, 1938). Kodama (1937) found similar effects in air at high pressure, Behnke, Forbes and Motley (1936) reported that in addition to causing a contraction of visual fields, OHP induced pupillary dilatation. This change might, conceivably, be interpreted as arising from some effect of OHP on the autonomic nervous system, from a release of humoral substances into the circulation, or from a local action on the effector itself. In order to investigate these possibilities, sphincter and radial muscles of beef iris were exposed to  $O_2$  at pressures of from 5 to 6 atmospheres (Bean and Bohr, unpublished data), such exposures caused a reversible decrease in tonus. It was concluded therefore that the central nervous or hematogenous connections are not essential to the occurrence of the pupillary dilatation induced in animals on their exposure to OHP, although in all probability nervous and hematogenous, as well as local effects of OHP, contribute to the induction of that response.

**Intestinal muscle** Further experimental evidence bearing on the question of the site of action of OHP on smooth muscle is found in studies on isolated rabbit intestine (Bean and Bohr, 1940), the exposure of longitudinal duodenal muscle to  $O_2$  at about 75 pounds' gauge pressure resulted in a progressive loss in tonus, a decrease and irregularity in the amplitude of the spontaneous rhythmic contractions, and a decreased frequency of this rhythm to a point of periodic cessation interspersed by spasmodic unsustained increase in tonus. Pyloric sphincter tonus was also diminished by OHP. These tonus changes in both longitudinal and sphincter muscles were reversed by decompression.

Atropinization of isolated duodenal and pyloric sphincter muscle preceding compression failed to alter the response to OHP. It was inferred, therefore, that the peripheral influence of OHP on smooth muscle is due neither to a stimulation of intrinsic nerve fibers nor to an involvement of acetylcholine but rather

to some more direct action on the effector cells themselves, the possibility, however, that effects may be mediated by other means in the intact animal was not dismissed. Tests of the bath solution showed no increase in lactic acid.

The response of longitudinal duodenal muscle to OHP was found to be so very similar to that induced by low  $O_2$  tensions and by cyanide as to suggest that it is caused by conditions approximating those of anaerobiosis arising from a poisoning of respiratory enzymes. But the finding (Bean and Bohr, unpublished data) that OHP caused a sharp drop in the tonus of the pyloric sphincter (rabbit) whereas cyanide induced little or no change in that tissue, indicates that the mode of action of OHP is not identical with that of cyanide and that it must involve some other mechanism than the cyanide sensitive cytochrome-oxidase enzyme system. A comparison of the effects of OHP on longitudinal duodenal muscle and on pyloric sphincter showed that while the tonus of both tissues was decreased by OHP, the tonus of the sphincter was much more markedly and rapidly depressed than was that of the longitudinal duodenal muscle. Incidentally, this difference in the reaction to OHP, together with the inverse effects induced in these same tissues by cyanide, is highly suggestive that the maintenance of tonus in these two tissues is dependent upon different enzyme systems.

*Striated muscle and nerve* Since sectioning of the motor nerves relaxed the spastic muscular contractions of  $O_2$  convulsions, Bert concluded that the seizures were not caused by the action of the  $O_2$  on the muscles themselves. He also observed that striated muscle in frogs which had succumbed to  $O_2$  at pressures of 3 atmospheres were responsive to direct artificial stimulation, although spinal reflexes could no longer be elicited. In frog muscle-nerve preparations suspended for 26 hours in hyperoxygenated air at a pressure of 15 atmospheres, it was found that the excitability of the nerve (as determined by muscle reaction) was lost, yet the muscle itself was still reactive to direct stimulation. These results suggest that striated muscle is relatively more resistant to the adverse action of increased  $O_2$  pressure than is the C N S or nerve fiber, but they do not rule out the myoneural junction as the possible vulnerable point of attack on the nerve-muscle preparation.

Valuable as Bert's experiments on isolated tissues are, they are very few in number because, as he said, the results of the few agreed so well with his previous expectations that a larger number was deemed unnecessary. His results may not truly represent the effects of OHP alone since his tests were made after rapid decompression of the preparations to normal atmospheric pressure following their exposure to very high pressures. Furthermore, in these, as in so many of Bert's experiments on  $O_2$  poisoning, highly compressed air or compressed hyperoxygenated air was used rather than pure  $O_2$ , on the assumption that the effects of compressed air are due only to the partial pressure of the contained  $O_2$ .

Hill and Macleod (1903c) performed several experiments on isolated striated muscle and nerve preparations in which the tissues were exposed to  $O_2$  at pressures as high as 50 atmospheres, no change from the normal was found in the contraction curve of the gastrocnemius muscle recorded under pressure, but the curve of the sartorius showed some slight alteration. These data, although

limited, again suggest that striated muscle and nerve are quite resistant to the deleterious action of OHP

The scanty data concerning the effects of OHP on isolated muscle and the methods by which they were obtained called for a more careful and extensive series of experiments Bean and Bohr (1938) using photographic methods carried out experiments on isolated striated muscle, muscle-nerve and reflex preparations of frogs It was found that exposure to  $O_2$  at pressures of about 5 atmospheres caused an initial increase in the height of contractions elicited by stimulating the muscle directly or through its nerve, subsequently, however, there occurred a slowly progressive decrease in height These effects were found to be only partially reversible The nerve fiber and the myoneural junction were apparently no more profoundly affected by the high  $O_2$  than was the muscle itself This toxic action of OHP was attributed to a poisoning of respiratory enzymes Isolated cardiac muscle has also been shown to be somewhat similarly affected by OHP (Bohr and Bean, 1939)

*7 Pathology* Although Bert was aware of the view that breathing  $O_2$  in high concentrations at atmospheric pressure caused lung damage, he made no extensive search for possible lung damage in his experimental animals which had been exposed to compressed hyperoxygenated air or to OHP In the rare examinations he did make he found neither congestion nor ecchymoses either in the lungs or in the nerve centers, but the eyes of a dog which had suffered convulsions on rapid decompression revealed hemorrhages.

In sparrows he found pathologic changes in the diploe and meninges which he likened to those seen in asphyxia He says, "On ne trouve ni congestions, ni ecchymoses, dans les pourchons et dans les centres nerveux. Seulement, d'une manière constante, chez les moineaux, on voit le diplôe crânien rempli d'un épanchement en piqueté, en taches plus ou moins grandes, ou même en nappe, envahissant toute la région occipitale, et, dans les cas les plus violents, toute l'étendue du crâne Ces suffusions sanguines, dont le mécanisme ne me paraît point facile à expliquer, sont constantes dans l'empoisonnement par l'oxygène Elles arrivent bien avant le moment de la mort Mais elles ne sont pas spéciales à ce genre de mort, et dans les expériences qui précèdent on les trouve signalées, même dans l'asphyxie simple, sous diminution de pression "

Bert's experimental procedures in his study of high  $O_2$  pressure effects were, with few exceptions, such that any pathology which he might have found could not be safely interpreted as having been caused by the  $O_2$  alone The decompression rates in his dog experiments were entirely too fast (many of them were abrupt "brusquement") to ensure the absence of embolism even if almost pure  $O_2$  had been the compression medium, to say nothing of his experiments in which the  $O_2$  content was only about 60 per cent Furthermore in most of these experiments the  $CO_2$  was permitted to accumulate in the respired gas, in one experiment the  $CO_2$  content of the respired gas reached 12.9 per cent Both of these complicating factors may have contributed to the pathology he described

The first report that OHP caused lung pathology is apparently that of Thompson (1889) A guinea pig and a dog which had had convulsions under  $O_2$  pres-



sure and had died a few minutes after decompression, showed at autopsy, "great pulmonary congestion" and "over distention of the right heart" The other viscera were "exsanguinated" The decompressions were carried out gradually thus eliminating possible complications from bubble formation

Smith (1897a, b) found that a mouse breathing  $O_2$  at pressures of about 2 atmospheres died within 24 hours, its lungs at autopsy were congested, consolidated and sank in water, the alveoli were almost completely filled with exudate, and the blood vessels were extremely congested These pathological changes Smith thought, occurred early in the exposure The higher the pressure the shorter was the time during which the lungs were able to withstand the effects of oxygen Smith claimed that exposure of animals to  $O_2$  at from 170 to 180 per cent of an atmosphere altered the lungs so that they could not "actively" absorb  $O_2$

In his later work Smith (1899) demonstrated that even at very moderately increased pressures of  $O_2$  the lungs become "inflamed"—effects which are reminiscent of the early reports of Lavoisier and Priestley In  $O_2$  at 128.9 per cent of an atmosphere mice became sluggish after 40 hours and died in 69 hours, the lungs at autopsy showed consolidation and congestion There was also congestion in the liver, spleen and kidneys The more characteristic pathology which Smith found from exposures to  $O_2$  at pressures of between one and two atmospheres as well as for higher pressures was as follows "The lungs were deeply congested, and sank in the fixing fluid Spleen slightly enlarged Other organs normal On microscopic examination, the tissues of the lungs showed intense congestion in the large and small blood vessels The alveoli were to a great extent filled with an exudate, which was granular and fibrillated in appearance, but did not give the fibrin stain by Weigert's method, nor with eosin The Weigert's stain showed one or two streptococci These, however, were exceedingly few in number, and as the mice died overnight in a somewhat warm atmosphere, their presence was probably accidental There were no leucocytes in the exudate The pneumonic condition was universal, and could therefore be compared only with the earliest stages of croupous pneumonia The exudate itself was probably the cause of the embarrassed respiration and the animal's death It is inconceivable that with inflammation so extensive, the animal could have survived until the process had developed farther" Mice exposed to  $O_2$  pressures at from 3 to 3.5 atmospheres became dyspneic and when taken from the chamber after about 10 hours died immediately without convulsions, the lungs showed these same characteristic changes

Smith's experiments led him to conclude that the action of OHP could be divided into two phases "The one consisting in the slowly developing inflammatory effect seen most prominently in the lung tissue The other a rapidly developing effect on the nervous tissue, which we may in the meantime describe as functional Both effects persist after the animals have been restored to ordinary air, and this, since it is frequently inconsistent with recovery, we may regard as indicating a profound change in the tissue cells" Smith emphasized that the transition to the pathological stage in the lung was imperceptible His claim that this lung damage temporarily protects the C.N.S. from the effects of

breathing OHP, not substantiated by some more recent investigators, has been misinterpreted by some O<sub>2</sub> therapists as an argument that a lung suffering from pneumonia is less susceptible to the damaging effects of increased O<sub>2</sub> tensions than is the normal lung.

Hill and Macleod (1903c) confirmed the findings of Thompson and of Smith. They described changes which in the early stages were characterized chiefly by congestion of the alveolar capillaries but later hemorrhagic exudation and consolidation were present. These authors say, "To the naked eye the lungs present in the early stages a suffused redness. Patches of more intense exudation occur in the apices and edges of the lungs. At a later stage the congestion passes into typical hepatization, the lungs sink in water and are of a dark purple colour. The pneumonia is patchy if quickly, and universal if slowly developed." In prolonged exposure to O<sub>2</sub> at pressures of from 3 to 5 atmospheres, lung changes such as described by Hill and Macleod were observed in anesthetized dogs (morphine and urethane) by the reviewer in 1929 and these together with the occurrence of convulsive seizures necessitated the use of relatively short exposures for the investigation of the problem then in hand. Phillips (1931) also found that exposure of animals to OHP caused pneumonia.

Shilling and Adams (1933) observed lung changes in animals which had been exposed to OHP, which they described as hyperemia, severe congestion and edema, hemorrhagic exudation, transudation, and acute hemorrhage into the alveoli and tissue spaces. Some lungs were almost solid from hemorrhage and sank in water. The microscopic studies of the brain and nervous tissue were negative. The authors were convinced that lung damage was not the cause of the convulsive seizures because there was "practically no gross lung damage in animals autopsied immediately after the first convulsion occurring early during exposure to high pressures of oxygen. In several instances animals went on to death under high-pressure oxygen with lungs that were not sufficiently damaged to account for convulsions due to asphyxia."

These experiments of Shilling and Adams, directed toward a study of the use of OHP in the decompression of divers, led those authors to the opinion that men exposed to increased tensions of O<sub>2</sub> would, in all likelihood, have early subjective warning of impending lung damage and convulsive seizures because of the probable irritation of nasal and pharyngeal tissue together with general restlessness. It was thought that "Even after a severe convulsion, recovery would probably be rapid and complete if the exposure were immediately terminated." Behnke, Shaw, Shilling, Thompson and Messer (1934) observed pulmonary congestion and varying degrees of atelectasis, but "hemorrhage or edematous exudation into the alveoli or bronchi did not occur," in anesthetized (barbiturate) dogs exposed to OHP.

The findings of Hederer and Andre (1940) are in essential agreement with those described by earlier investigators and these authors maintain that the pathology induced by OHP is the same as that induced by prolonged exposures to hyper-oxygenated air or to pure O<sub>2</sub> at atmospheric pressure.

Ozono de Almeida (1934) found that one exposure to OHP rendered an animal

more susceptible to both the C N S effects and the lung damage of OHP in subsequent exposures. In addition to lung pathology it was also reported that rats exposed to OHP showed atrophy of the testes, destruction of seminal epithelium and disappearance of all epididymis spermatozoa, the epididymis, seminal vesicles and the prostate, however, were unaffected. Numerous castration and basophilic cells were found in the anterior hypophysis. The histologic alterations were reported to be similar to those produced by radium or x-rays. The resulting sterility in the male was irreversible. The female rat was less profoundly affected than the male and the sterility induced was transient, pregnancy was not interrupted, and the growth of new-born rats was apparently normal. It was further pointed out that previously docile rats became combative and hyperexcitable after their exposure to OHP.

This change to belligerency and hyperexcitability following exposure to OHP was also observed by the reviewer (1940, unpublished data) but in addition to this it was found that these rats, and especially those which had suffered convulsive seizures, retained a pronounced motor dysfunction predominant in the forelegs, which persisted for months. A subsequent study of the phenomenon in a long series of animals (Bean and Siegfried, 1943) revealed that this condition can be regularly induced in both fore and hind limbs and the general body musculature by successive short exposures to OHP over a period of several days. In some animals this motor paralysis has been produced without the occurrence of convulsive seizures. The extent and degree of involvement depends not only on the intensity of the exposure, i.e., the duration,  $O_2$  pressure, frequency and total number of successive exposures, but also on a rather striking individual variation in resistance and recovery. The fact that this condition has now persisted in survival animals for more than a year after the last exposure without significant abatement, is sufficient reason to believe that it is permanent.

The general features of this dysfunction, particularly the spastic nature of the paralysis, are highly suggestive of pathology involving the upper motor neurone. While the C N S phase of oxygen poisoning may often appear to be "functional", as Smith (1899) maintained it was, there can be little doubt but that OHP acts on the C N S in more than just a "functional" manner, it leaves a mark on the C N S which persists for long periods and which may be made indelible, particularly with successive exposures. A systematic examination of the C N S of animals in which this permanent motor dysfunction has been induced by OHP is in progress at the time of writing and while the evidence at hand does not as yet justify a final conclusion, the material obtained from preliminary experiments has shown definite pathology characterized as a "softening of the white matter" in the C.N.S. of animals in which this permanent motor dysfunction has been induced.

Turning to a consideration of the evidence of pathological action of OHP on the human organism, one finds it, naturally enough, less voluminous and less direct than that derived from animal experiments. Phillips (1931) reported that in the Davis Submersible Decompression Chamber "no evil effects, except subsequent sleepiness" were produced by exposures of men to  $O_2$  at a pressure of 2

atmospheres, in one case functional albuminuria was prevented by such exposures

Smith (1899) maintained that the autopsy findings in fatal cases of caisson sickness in men were so similar to those observed in his experimental animals poisoned by increased  $O_2$  pressure as to justify the belief that  $O_2$  poisoning contributed to the occurrence of caisson sickness. This view has found no very substantial support. Nevertheless, where the exposures to very high air pressures are prolonged, an involvement of an  $O_2$  poisoning in compressed air illness is not only possible but very probable, particularly in view of the additive effects of  $CO_2$ , high tensions of  $N_2$  and  $O_2$ , as discussed above (see section II). Furthermore the case reports of Shilling and Willgrube (1937) on divers who, having had attacks of caisson sickness, were placed in the recompression chamber and exposed to relatively high air pressure for considerable length of time, indicate that pulmonary pathology was induced by increased  $O_2$  pressure. Jenkinson (1939) reported that at a pressure of 4 atmospheres  $O_2$  is rapidly fatal.

The prediction voiced by Shilling and Adams (1933) that in men, recovery from  $O_2$  convulsions would probably be rapid and complete, finds some substantiation in the work of Behnke, Johnson, Poppen and Motley (1935). These authors report that in human subjects complete recovery from the effects of breathing OHP (syncope, absence of radial pulse and convulsions) "followed immediately" the inhalation of air. On the basis of their experimental results these investigators were led to the opinion that "Healthy men between the ages of 22 and 40 can breathe pure oxygen with comparative safety as follows: 4 hours at 1 atmosphere, 3 hours at 2 atmospheres, 2 hours at 3 atmospheres." Substernal pain and dry cough, however, were observed as a result of some exposures, this was interpreted as indicative of lung irritation. The pulmonary changes, however, were considered "not impressive" and of minor importance in comparison with the changes referable to the nervous system. But the authors do suggest that incipient pulmonary changes at 3 atmospheres' pressure may have been responsible for the attendant leucocytosis even though symptoms of pulmonary irritation such as cough and pain in the chest were absent. One subject was unconscious for 20 minutes after the onset of convulsions, this could hardly be termed an "instant" or an "immediate" recovery which Shaw, Behnke and Messer (1934) have stressed is a characteristic feature of  $O_2$  poisoning, and must represent a lag in tissue recovery.

In later experiments, Behnke, Forbes and Motley (1936) found a state of impending collapse in their subjects after 3 to 4 hours' exposure to  $O_2$  at 3 atmospheres and although consciousness was retained, all of them looked dazed and gave indication of partial stupefaction. The authors conclude that healthy men can breathe  $O_2$  at a pressure of 3 atmospheres (30 lbs gauge) for 3 hours without distressing symptoms. In this connection the authors report a "gradual recovery" of from 20 to 60 minutes in the subjects on their return to air at atmospheric pressure, nausea and dizziness disappeared within a few minutes but the return to the normal of blood pressure, pulse rate, pupillary diameter, visual acuity and facial color occupied a "considerably longer time." End and Long

(1942) accept the interpretation that  $O_2$  at a pressure of 3 atmospheres can be safely inhaled for 3 hours but believe that at 4 atmospheres  $O_2$  is dangerous

The symptomatic indications of damage induced in human lungs by exposure to OHP, "unimpressive" as they may be, cannot be dismissed as insignificant, this would appear to be especially so in view of the well-demonstrated pathology produced in experimental animals by such exposures and the observation that this pulmonary pathology develops gradually. The fact that an individual exposed to these adverse environmental conditions may manifest no striking reaction until a certain critical level of intensity or duration of exposure is reached, does not justify the assumption that his tissues remain entirely unaffected by anything short of that level. Actually an exposure carried to the critical level represents a breakdown test and it would be erroneous to maintain that no important changes occur except in those exposures which precipitate violent reactions and gross symptomatology of tissue damage. Autopsy examination might be surprisingly revealing.

Furthermore symptomatic recovery from the effects of OHP, while seemingly suggestive of an absence of pathology, may be misleading because of wide anatomical and physiological margins of safety. The possible dangers inherent in sub-symptomatic exposures to OHP can not, therefore, be ignored. On the other hand it must be recognized that under certain exigencies the administration of OHP, like that of  $O_2$  at atmospheric pressure, may not only be advisable, but may actually amount to a life-saving measure.

8 (a) *OHP in decompression from compressed air* The use of OHP has found practical application in facilitating decompression of men from compressed air. Bert had reported that breathing a gas mixture high in  $O_2$  content permitted decompression of animals with less danger of nitrogen bubble formation. About a quarter of a century later von Schrötter (1904) advocated that compressed air workers should breathe pure  $O_2$  for five minutes in order to wash out the nitrogen dissolved in the tissues before decompression. But Ham and Hill (1905), finding cats were convulsed in six minutes by  $O_2$  at pressures of 50 pounds, claimed it was "unsafe to use Schrötter's method of 50 pounds and upwards. Below that pressure the risks of fatal air embolism are much less and the method is hardly worth employing." Hill and Greenwood (1907) were of the same opinion. Zuntz (1909) recognized the feasibility of using  $O_2$  under increased pressure to aid in decompression as did Bornstein (1910). The early argument of Ham and Hill against the use of OHP in decompression of caisson workers was later changed following experiments of Twort and Hill (1912) on men, in which  $O_2$  was breathed for 9 minutes at a pressure of 3 atmospheres and during the subsequent decompression to atmospheric pressure. It was concluded from these experiments that the nitrogen dissolved in the urine under compressed air is rapidly cleared out by breathing OHP and the authors state "The practical application of these results to the prevention of caisson sickness is obvious." In more recent years many investigators have studied and recognized the advantages of using OHP in decompression (Hill, 1932, Shilling and Adams, 1933, Phillips (1931), Dorello, 1934, Davis, 1935, Behnke and Shaw, 1937, Jones et al., 1940, and others).

(b) *OHP in therapy* There is good reason for supposing that OHP might be of distinct value for therapeutic purposes in some conditions. Obviously such use calls again for an evaluation of possible benefits to be derived, and the disadvantages and dangers to be encountered. One of the earliest attempts to use OHP therapeutically is that of Valenzuela (1887) who had found in preliminary animal experiments (rabbit) that exposure to  $O_2$  at pressures as high as about two atmospheres (1520 mm Hg) for an hour caused a fall in the body temperature from  $38.4^\circ C$  to  $32.2^\circ C$ . The general effect of such exposure was said to produce a torpor in the animals from which they recovered on decompression. He then studied the effects of OHP on febrile septicemia induced in a rabbit by the inoculation of "serous fluid with a putrid smell from a dead body". The temperature of septicemic animals treated by exposure to OHP ( $O_2$  at pressures of 1.4 atmospheres for 2 hours) decreased  $1.7^\circ C$  whereas in the controls without treatment it increased  $3^\circ C$ . After 3 successive exposures on 3 days the  $O_2$  treated rabbit had recovered on the fourth day, the control was dead. Valenzuela then applied the same treatment to human subjects suffering from pneumonia and reported beneficial results.

Fischer and Andersen (1926a, b) demonstrated that the cells of some tumors were more susceptible to the destructive action of OHP than normal cells, but attempts to use this differential susceptibility to destroy tumors in vivo (Fischer, Andersen, Demuth and Laser, 1927) were not very successful. Of 158 tumorous mice exposed to  $O_2$ , 2 were completely cured and 3 were favorably affected, 68 of the animals, however, were killed by the  $O_2$  treatment. In other work on this subject (Fischer, Andersen and Demuth, 1926) it was found that if copper or selenium were injected previous to the exposure to the  $O_2$ , the selective destruction of tumor cells was more pronounced.

Ozorio de Almeida (1934b) found that the selective destruction of tumor cells by OHP was enhanced by previously starving the animals, the starved animals became more resistant to the OHP whereas the tumor cells were completely destroyed. Basset et al. (1935) reported that mouse sarcoma 37, bathed in physiological saline, was destroyed by exposure to  $O_2$  at a pressure of 1800 atmospheres for 30 minutes but at pressures of 1000 atmospheres it was not so affected. Campbell (1937) failed to obtain any satisfactory destruction of rat tumors by OHP. Attempts to use OHP in the treatment of malignancies in man by Auler, Herzogenroth and Wolff (1929) and by the South American investigators have not met with any notable degree of success. The treatment of leprosy by OHP has also been carried out in South American laboratories and while it appeared that the progress of the disease may have been somewhat slowed thereby, no cure resulted. Ozorio de Almeida and Pacheco (1941) employed OHP in therapeutic studies of experimental gas gangrene, Pacheco and Costa (1940, 1941) carried out experiments on the influence of OHP on clostridium welchii, de Mesquita (1941) reported cases of psoriasis treated by OHP.

One point in connection with the possible application of OHP to therapy which is deserving of special mention concerns the influence of  $CO_2$ . It is now well recognized, after repeated demonstration by various investigators that the

adverse effects of OHP are increased to a striking degree by the presence of  $\text{CO}_2$ . Other things being equal, then, one would expect that in those conditions in which there is an abnormal retention of  $\text{CO}_2$  the adverse reactions to OHP might be unduly prominent. While it would seem that OHP should readily relieve hypo-oxemias, such for example as that resulting from extensive pulmonary pathology, such treatment might at the same time be complicated by the failure to concomitantly facilitate the removal of  $\text{CO}_2$ . It is not unlikely that some other types of acidosis might also augment the adverse effects of OHP.

(c) *OHP in CO poisoning* The administration of  $\text{O}_2$ - $\text{CO}_2$  mixture for the resuscitation from carbon monoxide poisoning, long championed by Henderson, has proven of value, but the use of OHP in the treatment of such poisoning might seem to offer some further advantages. Haldane (1895) pointed out that the higher the tension of  $\text{O}_2$  to which an animal is exposed the less dependent it is on its RBC's for oxygen, because of the increased amount of  $\text{O}_2$  carried as simple solution in the plasma. The importance of this dissolved  $\text{O}_2$  is further enhanced by the fact that the presence of CO in the blood so alters the  $\text{O}_2$  dissociation curve of hemoglobin as to render that fraction of the hemoglobin which may still be functional, less effective in the carriage of  $\text{O}_2$  than it otherwise would be. OHP should, then, be especially effective in preventing the anoxia in CO poisoning.

In addition to preventing anoxia in CO poisoning, the use of OHP would seem to provide further advantage in that it should be more effective in displacing carbon-monoxide from its combination with hemoglobin and thus for two reasons: first, simply because at high concentrations  $\text{O}_2$  can more readily compete with CO for hemoglobin; second, because the increased blood acidity obtaining in animals exposed to OHP (Bean, 1931) should, according to Stadie and Martin (1925), facilitate the removal of CO from the hemoglobin.

In experimental tests Haldane (1895) reported that OHP ( $\text{O}_2$  at pressures of about 2 atmospheres) abolished the usual toxic action of even very high concentrations of CO. He further found that animals whose hemoglobin had been saturated with CO and whose lives had been maintained under those conditions by OHP, could be safely returned to the normal atmosphere if the CO were first washed out without permitting the  $\text{O}_2$  pressure to fall below 2 atmospheres. These experiments were performed as part of an investigation of mine rescue methods, but no attempt seems to have been made at that time in their practical application to resuscitation from CO poisoning. The report of Schmidt-Kehl (1926) also points to the possible use of OHP for this purpose.

One reason for using  $\text{CO}_2$  along with  $\text{O}_2$  at atmospheric pressure in the treatment of CO poisoning is that it prevents an excessive alkalinization of respiratory control mechanisms which would otherwise occur as the result of the release of Na from lactate which had accumulated during the period of anoxia. Another advantage of the use of  $\text{CO}_2$  is that it should, according to the findings of Stadie and Martin (1925), facilitate the removal of the CO from its combination with hemoglobin. Both of these arguments would appear to be applicable also to the use of OHP in the treatment of CO poisoning. But if, as has been observed (Bean and Haldane, 1932), the lactic acid is not as adequately removed in OHP as

under normal conditions, the release of Na from the lactate on the administration of OHP may be of less significance than in the case of  $O_2$  administration at atmospheric pressure. On the whole the possible use of OHP in carbon monoxide poisoning seemed to warrant further investigation. Experiments combining the administration of OHP and artificial respiration were therefore carried out by Doctor Bohr and the author (unpublished data, 1941) on rats. Circumstances prevented the completion of that study so that the data were insufficient to justify a final conclusion, but the results were suggestive of beneficial effects.

End and Long (1942) found that  $O_2$  at pressures of about 30 pounds would resuscitate dogs and guinea pigs poisoned by carbon monoxide even without artificial respiration. The curves representing the elimination of carbon-monoxide from the hemoglobin, however, became asymptotic with about 20 per cent of the hemoglobin still combined with CO, this "flattening out" was quite a constant finding and the authors suggest it may have been "due to the animals' habit of falling asleep. The diminished respiration and more sluggish circulation incidental to sleep evidently retard elimination of carbon monoxide." It was proposed that OHP be used for resuscitation of human beings poisoned by CO.

(d) *OHP in shock* Another condition in which the therapeutic use of OHP might conceivably be of benefit is that of shock. Following severe operative procedures, such as that of decerebellation which is occasionally attended by considerable loss of blood, it has been customary for the reviewer to subject the operated animal (pigeon and rat) to  $O_2$  at pressures of about 2.5 atmospheres in short intermittent exposures. The apparent beneficial results derived from this treatment in tiding the animals over post-operative depressive states, augurs well for its possible success in the treatment of some cases of secondary shock. Frank and Fine (1943), however, report that exposure of experimental animals to  $O_2$  at a pressure of 3 atmospheres did not favorably alter either the survival time or any of the commonly observed phenomena of shock, even in those animals (7 of 15) in which the  $O_2$  concentration of venous blood was thereby maintained at normal levels by the OHP, these authors concluded that oxygen as a therapeutic agent in hemorrhagic shock is "of doubtful value."

In attempts to use OHP therapeutically in any of the conditions for which it might appear to promise advantages, its inherent dangers, already emphasized, must be recognized. Furthermore, in experimental studies, due cognizance must be taken of the fact that OHP, improperly employed may result in deleterious effects which may completely overshadow any possible benefits and lead to an unfair condemnation of a treatment which if administered with proper precaution might be of distinct value.

ETIOLOGY OF THE REACTIONS TO OXYGEN AT HIGH PRESSURE. Bringing together the various interpretations offered in explanation for the adverse effects induced by OHP calls for a consideration of the experimental conditions and procedures which led to the original conclusion that OHP was toxic and caused convulsions and death of animals exposed to it.

In his pressure studies, Bart analyzed the air of closed chambers in which birds



had succumbed as a result of the accumulation of their expired  $\text{CO}_2$ , an adequate  $\text{O}_2$  tension having been provided either by the use of hyperoxygenated air or by ordinary air at moderately increased pressures. From the results of these experiments he concluded that death occurred when the  $\text{CO}_2$  tension of the chamber air had risen to a relatively constant value, which for sparrows was about 26 ( $\text{CO}_2$  percentage  $\times$  pressure in atmospheres). It was observed, however, that sparrows exposed to higher pressures succumbed before the  $\text{CO}_2$  tension had risen to the theoretically lethal level, in fact, for pressures above 3 atmospheres, the  $\text{CO}_2$  tension in the chamber at the time of death progressively decreased, as the barometric pressure employed was increased (See tables 5 and 6.) From this Bert concluded that death in these higher pressures could not have been caused

TABLE 5

1	2	3	4	5	6	7	8	9	10
NUMÉROS DES EXPÉRIENCES	PRESSION	DURÉE DE LA VIE	DURÉE DE LA VIE POUR UN LITRE D'AIR A 76 C	TENSION PRIMITIVE DE L'OXYGÈNE	COMPOSITION DE L'AIR MORTEL		$\text{CO}_2 \times P$	$\text{O} \times P$	$\frac{\text{CO}_2}{\text{O}}$
					$\text{CO}_2$	O			
	atm	h m.	h m						
CXXXII	2	3 49	3 4	41 8	12 6	3 2	25 2	6 4	0 72
CXXXI	3	1 50	1	62 7	7 8	10 7	23 4	32 1	0 75
CXXVII	4	1 35	39	83 6	5 6	13 2	22 4	52 8	0 72
CXXIII	5½	1 30	27	120 1	3 8	15 5	21 8	89 1	0 70
CXXI	6	1 20	22	125 4	3 5	16	21 0	96	0 71
CXXVIII	8	1 38	20	167 2	2 4	16 8	19 2	134 4	0 60
CXXIV	9	1 10	14	188 1	2	17 5	18 0	157 5	0 59
CXXV	12	45	6	250 8	1 2	18 5	14 4	222 8	0 50
CXXX	12	45	1	250 8	1 3	18 7	15 6	224 4	0 59
CXXIX	14	45	1	292 6	0 9	18 5	12 6	263 2	0 43
CXXXI	14	39	4	292 6	0 9	18 5	13 2	263 2	0 43
CXXVI	15	39	1	313 5	0 8	19 4	11 2	291	0 53
CXXXIII	17	39	3	355 3	0 6	18 8	10 2	319 6	0 30
CXX	20	25	2	418 0	0 4	18 8	8	319 6	0 30

by the accumulating  $\text{CO}_2$ , and that it must, therefore, have been due to the increased  $\text{O}_2$  tensions.

Certainly these particular data of Bert are highly suggestive that the increased  $\text{O}_2$  tension was involved in the cause of death at the increased pressures and that  $\text{O}_2$  at high pressures might, therefore, as he says, be considered a dangerous agent. But they would hardly seem to justify the conclusion that the increased  $\text{O}_2$  by itself was the only lethal agent. At any rate our present knowledge of the interrelationship of  $\text{CO}_2$  and high pressures of either air or  $\text{O}_2$ , does not permit the dismissal of the  $\text{CO}_2$  tensions in these experiments of Bert as insignificant, even though some of them were far below his theoretically lethal constant. Nevertheless it was these data upon which Bert based his conclusion that while at moderately increased barometric pressure an accumulation of  $\text{CO}_2$  in the pressure chamber might contribute to the death of the animals, at the higher pres-

sure death was caused exclusively by the increased  $O_2$  tension alone. Speaking of the effects of ordinary air at increased pressure in a chamber without either ventilation or  $CO_2$  absorption, Bert says "Pour les pressions très-élevées, la mort est due exclusivement à la tension trop considérable de l'oxygène ambiant."

Bert experienced considerable difficulty, as he confesses, in devising suitable means for removing  $CO_2$  from the compression chamber of his sparrow experiments. This, together with his earlier conclusions regarding the lethal  $CO_2$  tension, may help to explain why he seemingly dismissed the removal of  $CO_2$  from respired air as unimportant. He did, however, perform a few experiments

TABLE 6

1	2	3	4	5	6	7	8	9	10
NUMÉROS DES EXPÉRIENCES	PRESSION BAROMÉTRIQUE	TENSION D'OXYGÈNE DANS LE MÉLANGE PRIMITIF	QUANTITÉS CORRESPONDANTES D'ATMOSPHÈRES D'AIR	DURÉE DE LA VIE	DURÉE DE LA VIE POUR UN LITRE D'AIR À 76 C	COMPOSITION DE L'AIR MORTEL		$CO_2$ X F	PHÉNOMÈNES GÉNÉRAUX PRÉSENTÉS PAR LES ANIMAUX
						$CO_2$	O		
CXLIII	1 25	32 5	1 5	1 30	1 36	22 1	3 5	27 6	Pas de suffusions crâniennes
CXLIV	1 5	69 0	3 3	1 30	1 36	16 7	23 6	25 1	Pointillé rouge au crâne
CXLVI	2 5	115 5	5 5	3	53	11 1	33 3	27 7	Suffusions crâniennes
CXL	2 0	117 6	5 6	3	53	13 4	44 4	26 8	Suffusions crâniennes
CXXXIX	1 75	146 3	7 8	2 20	31	11 9	67 8	20 8	Suffusions crâniennes
CXXXVIII	3 0	238 0	12 1	1 15	10	5 6	78 9	16 8	Suffusions crâniennes
CXLI	4 0	501 6	14 4	1	7	2 1	71 1	8 4	Convulsions suffusions
CXXXVII	5 0	415 0	19 7	1 20	6	1 4	80 5	7 0	Convulsions suffusions
CXLII	8 5	433 5	20 7	20	2	0 8	47 8	6 8	Convulsions suffusions
CXLV	5 5	467 5	22 3	20	1	1 0	82 5	5 5	Convulsions suffusions

on birds in which the  $CO_2$  was removed by partially filling the compression chamber with a potash solution. In these experiments, in which he says all trace of  $CO_2$  was removed from the chamber, the death of those birds which did not succumb because of a depletion of the  $O_2$ , was assumed to have been caused by the toxic action of the increased  $O_2$  tension of the highly compressed air. But such an assumption of an unadulterated  $O_2$  effect cannot be accepted without some reservation, the very high barometric pressure of air employed must certainly have introduced complicating factors not attributable to the increased  $O_2$  tension by itself.

As one reviews Bert's experimental protocols he becomes increasingly impressed with the apparent disregard which that investigator had for factors we now know are exceedingly important variables in, if not determinants of, the

response to increased pressures This apparent disregard is perhaps best summed up in his statement to the effect that all the influences which changes in barometric pressure may exert on animals are due either to an insufficiency or to an excess of  $O_2$  tension ("*Pas assez d'oxygène, en tension, ou trop d'oxygène, toute l'influence que les modifications barométriques exercent sur les animaux se résume en ces termes*") His experiments on oxygen poisoning in dogs bear witness to the strength of his conviction on this point and deserve special mention

These experiments were, with few exceptions, carried out as rebreathing procedures in which no attempt was made to absorb the expired  $CO_2$  accumulating in the relatively small rebreathing balloon In the majority of these experiments the  $CO_2$  of the gas mixture breathed at increased pressure was between 8 and 10 per cent, the lowest figure given for this type of procedure was 5.4 and in one case it reached the astounding value of 12.9 per cent (at 6.75 atmospheres, the pressure used in that particular experiment, this would represent a tension of 87 per cent of one atmosphere) For those few experiments in which attempts were made to remove the  $CO_2$  by pieces of potash placed in the bottom of a bottle, or by potash solution in the rebreathing balloon, no  $CO_2$  analyses of the gas rebreathed are given This removal of the  $CO_2$  was, he insisted, quite unnecessary for the reasons presented above

In addition to this matter of  $CO_2$ , Bert's experiments on dogs are open to question on another score, to which brief reference has already been made, viz., that of the very rapid decompression rates which were used In spite of the fact that the pressures employed were as high as 8 atmospheres, and that the  $O_2$  content of the compression medium was as low as 60 per cent, the decompression was often abrupt Such procedures must have frequently resulted in  $O_2$  and  $N_2$  emboli which in themselves might well precipitate convulsive seizures and significantly enough the convulsions in Bert's dogs occurred during or following decompression These seizures in dogs, described by Bert as  $O_2$  convulsions were as Hill and Macleod (1903c) pointed out "clearly decompression results, and due to the effervescence of oxygen gas in the central nervous system"

In summary then it may be said that, important as the investigations of Bert were in that they drew attention to a new feature of the adverse effects of increased tensions of  $O_2$ , the experimental results which he details as representing the toxic action of OHP alone, are open to serious criticism on at least three main counts first, because of the gross failure to recognize the possible importance of very high  $CO_2$  tensions in the gas mixtures used, second, because of the frequent employment of very high barometric pressures of air or of high  $N_2$  percentage mixtures in order to attain the desired increase in  $O_2$  tension, third, because of the very rapid decompression employed In considering Bert's views then it may be well to keep in mind that many of the reactions he described as those of  $O_2$  poisoning involve more than the effects of  $O_2$  alone The fact that in spite of Bert's intentional disregard of these complicating experimental conditions, his observations have on the whole been confirmed by subsequent investigations which are not open to these criticisms, is more than of passing interest for it points to the possible significance of etiological factors discussed below

A consideration of the accumulated data, including that of Bert, provides abundant evidence for concluding that the effects of exposure to OHP are generalized and involve many if not all tissues of the body and cannot, therefore, be legitimately localized to any one single or even several tissues, to the exclusion of others. But the outstanding features of the manifestations of those effects are predominantly those of respiratory difficulty, motor disturbances which frequently, though not invariably, include convulsive attacks, and eventual death. These features have directed attention to the lungs and to the C.N.S. which have been considered as organs for which the adverse action of OHP has an especial predilection and constitute the background for Smith's conclusion (1899) that the cause of the reactions to OHP are (1) pulmonary pathology and (2) an involvement of the C.N.S. If the proviso of admitting certain qualifications be permitted no strenuous opposition can be reasonably voiced against this interpretation so far as it goes and it provides a basis upon which the more intimate etiology of the responses to OHP may be discussed.

1 *Pulmonary Pathology as an Etiological Factor* Thompson (1889) observing extensive pulmonary pathology in animals which had been exposed to OHP and had suffered convulsions therein, proposed that such pathology was an important contributor to the convulsive seizures. Smith (1899) finding dyspnea a prominent reaction in exposures to OHP, and at autopsy, severe pulmonary damage and pneumonia, was led to the belief that such pulmonary damage constituted a separate and a more slowly developing phase of  $O_2$  poisoning than that of C.N.S. involvement, and that while this pathology might tend to postpone the C.N.S. effects of OHP by slowing up the  $O_2$  absorption, it ultimately caused death of the animal.

Extensive pulmonary pathology might conceivably contribute to the reactions in animals exposed to OHP in two ways: 1, by preventing  $O_2$  absorption and thus leading to an hypo-oxygenemia, or 2, by interfering with the elimination of  $CO_2$ . Where the increased  $O_2$  pressure is very little above that of one atmosphere both of these conditions develop after prolonged exposures and the situation is the same as that when breathing hyperoxygenated air or pure  $O_2$  at atmospheric pressure for prolonged periods, so far as the tissues beyond the lungs are concerned the condition may be then essentially one of low, rather than increased,  $O_2$  tension as discussed above in section I. However, where the  $O_2$  pressure is in excess of several atmospheres the condition of hypo-oxygenemia would not be likely to occur so long as the pressure was maintained. On the other hand, if the pathology be at all extensive as it frequently is, there would be an interference with the removal of  $CO_2$ . This might be partially compensated for by the establishment of a new diffusion gradient through hyperpnea and the elevation of blood  $pCO_2$  so that with the attainment of a new steady state the elimination of  $CO_2$  might not be appreciably decreased as has been repeatedly demonstrated. But because even a relatively small increase in  $CO_2$  tension assumes a peculiarly high potency when associated with OHP any interference with the normal  $CO_2$  removal at the lung cannot be dismissed as insignificant. This is particularly so because the enhanced reactions ensuing from the addition of small amounts of

exogenous  $\text{CO}_2$  to OHP cannot be distinguished qualitatively from those induced by OHP in the absence of exogenous  $\text{CO}_2$

Stadie, Riggs and Haugaard (1944) dismiss the possibility that in exposures to OHP there is any retention of  $\text{CO}_2$  in the blood or tissues such as is observed in other types of pulmonary pathology. In support of this opinion they cite the report of Behnke et al (1934) that the arterial  $\text{pCO}_2$  was not increased in animals exposed to OHP. Now if the exposure to OHP be such as to induce no pulmonary pathology—and as already pointed out this is not infrequently the case—there would, of course be no reason to expect that  $\text{CO}_2$  elimination at the lungs would be very appreciably interfered with at pressures of 3 to 4 atmospheres, in fact its elimination might be facilitated under such conditions because of the hyperpnea which almost invariably occurs. Furthermore, one might by definition even rule out pulmonary pathology as a part of poisoning by OHP, since the typical reactions to OHP do occur in the absence of any demonstrable pathology, and in that case retention of  $\text{CO}_2$  because of disturbed elimination of  $\text{CO}_2$  at the lungs would also be ruled out. However, if pulmonary pathology be admitted as a part of the picture, the very extensive and severe lung damage frequently observed by so many investigators (see section on pathology) must, just as many other types of pulmonary pathology, lead to a retention of  $\text{CO}_2$ .

The importance of pulmonary pathology induced by exposure of animals to OHP was early impressed upon the reviewer performing experiments in which the effects of OHP on blood acidity, uncomplicated by this pulmonary pathology, were under study. The observations then and since have led to the conviction that in sacrifice experiments on animals exposed to OHP an inspection of the lungs for at least gross pathology should be made in the interpretation of results.

The mode of action by which OHP induces pulmonary pathology has not been clearly demonstrated. The more general view has been that OHP acts as an irritant directly on the pulmonary tissue. Ozorio de Almeida (1934) has interpreted the pulmonary pathology as a secondary result of diminished oxidations. Still another interpretation which has been offered is that it is secondary to circulatory effects of OHP, particularly on the right side of the heart. This last view is reminiscent of the explanation offered by Karsner for the pulmonary changes found in animals exposed to  $\text{O}_2$  at atmospheric pressure.

In summary then one may conclude that the pulmonary pathology induced by OHP does contribute while the animals are under the increased pressure to the reactions referred to as those of poisoning by OHP. This contribution is to be attributed to the interference with the normal removal of  $\text{CO}_2$ . During decompression and in the post decompressional period, those reactions which represent a persistence of the toxic effects of OHP induced during the maintenance of the increased pressure are further complicated by the pulmonary pathology which, with the animal's return to normal pressure conditions, results in hypo-oxemia. But the fact that respiratory reactions, blood changes, convulsive seizures and death commonly occur in animals exposed to OHP in the absence of any demonstrable pulmonary pathology (Bert, 1878, Thompson, 1889, Smith, 1899, Born-

stein and Stromk, 1912, Bean, 1929, 1931, Shilling and Adams, 1933) indicates that while pulmonary pathology induced by OHP is, when present, contributory, it is not essential to the induction of those reactions typical of poisoning by OHP

2 *The C.N.S. as the Site of Origin of the Reactions to Oxygen at High Pressure*  
Bert concluded that the origin of the more pronounced reactions to OHP, particularly the convulsive seizures, was the C.N.S. This conclusion was based on several observations: the relief of the seizures by chloroform anesthesia, the relaxation of convulsively contracted muscles by section of their motor nerves and the retention of the excitability of striated muscle to direct stimulation after their reflex excitability had been lost. Significantly enough convulsive seizures did not occur either in air at pressures much below 19 atmospheres or in  $O_2$  at pressures below about 3.5 atmospheres, that is to say, at pressures less than those necessary for the solution of 10 volumes percent of  $O_2$  in the plasma over and above the normal 20 volumes per cent held in combination with the hemoglobin.

It was this greater solution of the  $O_2$  in the plasma consequent upon exposure to high tensions of  $O_2$  which Bert maintained was a toxic agent for the C.N.S., so that when the  $O_2$  in the blood rose to about 35 volumes per cent it was rapidly fatal. "In mammals," he says, "trouble rapidly occurs only when the hemoglobin is saturated with oxygen and this gas enters the tissues from a state of simple solution." And "Whatever the explanation, it seems that for oxidation the tissues need borrowed oxygen, taken away from the oxyhemoglobin"—a comment especially apropos the importance and the carriage of  $CO_2$  by the blood in  $O_2$  poisoning discussed below. Because the convulsions continued after the pressure had been lowered, Bert contended that the real cause was some chemical change which, outlasting the apparent cause, continued to excite nervous tissue. That  $O_2$  at increased barometric pressure acts on the C.N.S. has been accepted by all subsequent investigators whose experimental data represent more truly the effects of OHP than do those of Bert. Williams and Beecher (1944) noted in *Drosophila* that the maintenance of balance and equilibrium was the first attribute to be permanently lost and were of the opinion that the primary effect of OHP in these forms was on the nervous system.

Shilling and Adams (1933) reported that extensive microscopic studies of nervous tissue in brains of animals subjected to  $O_2$  convulsions were made by Finley but that "the findings were essentially negative." Similarly Fahr (1941) recognizing that the symptoms of poisoning by OHP pointed to the C.N.S. as the site of involvement made special microscopic studies of the C.N.S. but no significant pathology was found there. The question arises as to whether the animals in these investigations may not have been sacrificed before degenerative changes could be demonstrated by the usual techniques.

Whether OHP itself operates directly on the C.N.S., or through the production of some intermediary toxic substance, or through a disturbed metabolism and removal of metabolites, or through a combination of these acting on peripheral as well as central structures, the permanently crippling motor dysfunction induced in rats by their repeated exposure to OHP (Bean and Siegfried, 1943) con-

stitutes conclusive evidence that OHP can cause permanent damage to the C N S and that therefore its influence is not entirely functional, as has heretofore been widely held. Recent work reveals degenerative changes in the C N S.

3 *Carbon Dioxide as An Etiological Factor* Although as already pointed out Bert dismissed CO<sub>2</sub> as of no consequence in the production of the reaction to O<sub>2</sub> at high pressure, an examination of his experimental protocols and data leaves no doubt but that CO<sub>2</sub> must have played a very large rôle in the response which he referred to as that of O<sub>2</sub> poisoning. The inverse relationship between the CO<sub>2</sub> and O<sub>2</sub> tensions in the lethal gas mixtures at various barometric pressures, shown above in tables 5 and 6, which formed the basis for Bert's conclusion that at high pressures the O<sub>2</sub> alone is responsible for the reaction, falls far short of ruling out CO<sub>2</sub> as an etiological factor. Indeed it would appear to be one of the earliest demonstrations of the now well recognized fact that tensions of CO<sub>2</sub> which at normal atmospheric pressure are too small to exert any very obvious effect, become highly and increasingly noxious when associated with an increased air or O<sub>2</sub> pressure.

The exceedingly high CO<sub>2</sub> content (as much as 12.9 per cent) of the gas mixture to which Bert's dogs were exposed has been mentioned above and, as would be expected, the blood of these animals showed a proportionately high CO<sub>2</sub> content. In one experiment, for example, the analysis of carotid blood in a dog when breathing air at normal pressure was CO<sub>2</sub> 22.3, O<sub>2</sub> 17.2 volumes per cent, but when breathing hyperoxygenated air (74 per cent O<sub>2</sub>, 10 per cent CO<sub>2</sub>) at 7.25 atmospheres pressure, the blood CO<sub>2</sub> had risen to 72.3 and the O<sub>2</sub> to 30.1 volumes per cent. In another experiment the blood CO<sub>2</sub> rose from 24.0 volumes per cent in air at normal pressure, to 92.5 volumes per cent in hyperoxygenated air at 6.75 atmospheres.

The blood gas analysis figures for Bert's experiments in which potash was used to absorb the expired CO<sub>2</sub>, are even more significant, in one such experiment with the animal in air at normal pressure the blood CO<sub>2</sub> was 20.9 and the O<sub>2</sub>, 19.8 volumes per cent, in hyperoxygenated air (88 per cent O<sub>2</sub>) at normal pressure the blood CO<sub>2</sub> was 34.5 and the O<sub>2</sub>, 20.9 volumes per cent, but at 6 atmospheres' pressure the CO<sub>2</sub> had risen to 63.5 and the O<sub>2</sub> to 26.3 volumes per cent. Increases in the CO<sub>2</sub> content of the blood such as these are significant. Hill and Macleod (1903c) point out that Bert's animals "must have been rendered comatose with CO<sub>2</sub>." Because Bert stated that at moderately increased O<sub>2</sub> pressures, the increase of CO<sub>2</sub> in the chamber did contribute to the adverse effects and death of the experimental subjects, his conclusion that CO<sub>2</sub> was of no consequence at higher O<sub>2</sub> pressures, is the more surprising.

Although Thompson (1889) indicated that the cause of the convulsions was undecided, he proposed that an accumulation of CO<sub>2</sub> in the body as a result of an interference with normal diffusion processes was responsible. "If the pressure of the inhaled atmosphere," he said, "be it oxygen or common air, is greatly increased, it is more difficult, and it requires more time for the CO<sub>2</sub> to diffuse from the blood into the air-vesicles, and from the air-vesicles into the tidal air in the wider portions of the air-passages. As a result, the CO<sub>2</sub> tends to accumulate in

the blood" Hill and Macleod (1903c) likewise claimed the diffusion of  $\text{CO}_2$  in the lungs was impeded in compressed gases

Evidence that  $\text{CO}_2$  might somehow be related to the action of  $\text{O}_2$  is found in the observation of Snell (1896) who maintained that an increase in  $\text{CO}_2$  from 0.045 to 0.1 per cent in caissons was the forerunner of much illness. Moir (1895) was of the same opinion and suggested that the air be washed free of all  $\text{CO}_2$  before it is pumped down to the men working in compressed air. Singstad (1936) recognizes the importance of  $\text{CO}_2$  in compressed air and maintains that the difficulty in breathing experienced by some divers at the greater depths is caused by excessive carbon dioxide in the helmet as a result of insufficient ventilation. The report of Yamada (1918) also points to the significance of  $\text{CO}_2$  in relation to the effects of  $\text{O}_2$ , he observed that 3 per cent  $\text{CO}_2$  in room air, if administered continuously to man, caused an hyperpnea which gradually decreased, but when that same concentration of  $\text{CO}_2$  was administered in pure  $\text{O}_2$  the hyperpnea increased.

Gesell (1923), in his study of the factors which contribute to the regulation of respiration through their influence on the intracellular acidity of the respiratory centers, pointed out that a failure of reduction of oxyhemoglobin should interfere with the normal function of the hemoglobin in the transport of  $\text{CO}_2$  from the tissues by the blood, this would lead to an accumulation of  $\text{CO}_2$  in the respiratory centers and a consequent increase in breathing. Such a failure in the reduction of oxyhemoglobin should theoretically take place when an animal is exposed to pure oxygen at pressures of about 3 atmospheres and above, for under that pressure the amount of  $\text{O}_2$  taken up by blood in simple solution would, according to physical laws, be more than sufficient to meet ordinary tissue requirements thus leaving the hemoglobin still fully saturated with  $\text{O}_2$ . The base normally provided by the reduction of oxyhemoglobin, and which normally serves for the transport of the greater part of the  $\text{CO}_2$ , would under such circumstances be missing and there should then follow, as a result, an undue accumulation of  $\text{CO}_2$  in the tissues. Gesell found that the addition of  $\text{CO}_2$  (5 per cent) to OHP (2700 mm Hg) caused rapid deterioration and death of rats in about one half hour, whereas exposure to either OHP (2700 mm Hg) without the  $\text{CO}_2$ , or to a mixture of  $\text{O}_2$  (300 mm Hg) and  $\text{N}_2$  (2400 mm Hg) with the addition of  $\text{CO}_2$  (5 per cent) produced little more than hyperpnea and restlessness. This enhancement of effects by the combination of  $\text{CO}_2$  with OHP was interpreted as evidence of an increased sensitivity of the organism to  $\text{CO}_2$  as a result of the disturbed transport of  $\text{CO}_2$ . But  $\text{CO}_2$  also augments the toxic action of OHP on organisms devoid of hemoglobin, as observed in some micro-organisms, and Williams and Beecher (1944) have shown that while *Drosophila* can withstand prolonged exposure to high  $\text{CO}_2$  tensions at atmospheric pressure (15 per cent) they are peculiarly sensitive to the presence of  $\text{CO}_2$  in the exposures to OHP. The authors state that  $\text{CO}_2$  facilitates the toxic action of OHP so that "the rate of poisoning can be described as a linear function of carbon dioxide tension."

According to the disturbed  $\text{CO}_2$  transport theory the effects of exposure to OHP should begin to be pronounced when the  $\text{O}_2$  tension reaches an equivalent of 3 atmospheres. Interestingly enough, the experiments of Bert (1878), Thomp-



son (1889), Smith (1899) and of subsequent investigators do show this to be true. Bert found that convulsions did not occur until the  $O_2$  tension of the compressed gas was somewhat in excess of 3 atmospheres, and that in those animals which succumbed, the blood during the exposure contained about 10 volumes per cent of  $O_2$  in simple solution in addition to the 20 volumes per cent combined with the hemoglobin. Under such conditions the oxyhemoglobin could not have been reduced and the animal must therefore have been deprived of one of its most important mechanisms of  $CO_2$  transport with the result of a partial damming back of  $CO_2$  in the tissues.

Bert pointed out that in  $O_2$  poisoning the oxyhemoglobin did not give up its  $O_2$  and his statement to the effect that for oxidation the tissues need borrowed oxygen, taken away from the hemoglobin, indicates that he had some notion that the failure of the oxyhemoglobin to give up its  $O_2$  might be an important factor in  $O_2$  poisoning. He says, "Or, fait du plus haut intérêt, c'est en présence de cet oxygène simplement dissous, libre, que les oxydations intimes se ralentissent, puis s'arrêtent. Il semble que les tissus aient besoin, pour s'oxyder, de l'oxygène emprunté, enlevé à la combinaison oxyhémoglobique, si bien que, en présence de l'oxygène dissous apporté par la compression, ou les tissus deviennent incapables d'opérer cette dissociation, ou les globules ne peuvent plus céder leur oxygène, et demeurent condamnés à la saturation perpétuelle." The decreased oxidation of which Bert speaks has been generally confirmed and is discussed more fully below, but one suggested explanation, which may be mentioned to advantage here is that it may be a secondary effect of the increased tissue acidity arising from a disturbed  $CO_2$  transport.

In order to test this theory of hemoglobin involvement in  $O_2$  poisoning a number of studies were carried out (Bean, 1929, 1931) among which were those on blood acidity, for if OHP disturbs the carriage of  $CO_2$  by the blood and causes a rise in tissue  $CO_2$ , these changes should be reflected in an alteration in blood pH. The changes in blood acidity of dogs exposed to OHP were therefore determined by continuous electrometric methods. The results showed that in those animals exposed to OHP for periods short of the convulsive stage, there occurred an increase in the acidity of both arterial and venous blood (pH changes of from 0.05 to 0.19) when the  $O_2$  pressure was increased to more than 3 atmospheres. Since, in these exposures the acid change was reversed on decompression it could not be attributed to lung damage, and under these conditions provides substantial evidence that the  $CO_2$  transport of the blood is disturbed by OHP. Such disturbance, resulting in an increased  $CO_2$  in the tissues, particularly those intimately associated with the regulation of breathing, provides an explanation for the hyperpnea and dyspnea which accompanied these exposures and which are characteristic findings in exposures of animals to OHP.

The observation in these same experiments that the elevation of blood acidity was less pronounced in those animals which were particularly hyperpneic during the exposure to OHP is worthy of note for it indicates that the increased acidity may be partially compensated by an increased breathing. Another compensatory mechanism suggested by these experiments is an increase in the volume flow of blood, although the recorded increase was only slight.

The continuous observation of the circulating venous blood in these experiments revealed that it remained in a highly oxygenated state (colour determination) throughout the exposure to OHP, withdrawn samples frothed copiously, indicating an  $O_2$  supersaturation. This proves unequivocally that under such  $O_2$  pressures the oxyhemoglobin is not reduced and it follows that  $CO_2$  transport must, as a result, be affected. The data, then, provide corroborating evidence that the acid change in the blood is to be explained on the basis of a disturbed  $CO_2$  transport, and an accumulation of  $CO_2$  in the tissues.

The experiments of Campbell (1929) provide perhaps even more direct evidence of an accumulation of  $CO_2$  in the tissues of animals exposed to OHP. Nitrogen was injected intraperitoneally and subcutaneously into rabbits which were then exposed to OHP. Analyses of the gas withdrawn from these artificial gas pockets of animals exposed to  $O_2$  at a pressure of 2639 mm Hg (3.5 atms) showed that the  $CO_2$  tension, as also that of the  $O_2$ , was markedly increased (average over 100 per cent) as compared with that from animals which were exposed to air in which the partial pressure of  $O_2$  was only 562 mm Hg. It was believed that the  $CO_2$  tension of the tissues was actually higher than that of the gas pockets. In those animals which succumbed to the increased  $O_2$  pressure the  $CO_2$  tension was distinctly higher than that of the survivors, "in four out of five animals the  $CO_2$  tension was 213 mm Hg (30 per cent) or more." Campbell points out that this accumulation of  $CO_2$  may be the cause of the narcosis and convulsions of  $O_2$  poisoning but suggests that the explanation of the increased  $CO_2$  may be a vasoconstrictive effect of  $O_2$  (Campbell and Hill, 1931).

Hill (1933) cites Campbell's results as indicative of a disturbed  $CO_2$  transport arising from the failure in the reduction of the oxyhemoglobin. In his own experiments Hill found that a preliminary 15 minute exposure to a mixture of 5 per cent  $CO_2$  and 95 per cent  $O_2$  at atmospheric pressure lowered the critical pressure at which convulsions occurred when the animals were subsequently exposed to OHP. The onset of convulsions was also found to be accelerated by  $CO_2$ , a monkey showed no symptoms of  $O_2$  poisoning in a 30 minute exposure to  $O_2$  at a pressure of 4.7 atmospheres but after a preliminary exposure to 5 per cent  $CO_2$  and 95 per cent  $O_2$  at atmospheric pressure, convulsions came on 18 minutes after raising the pressure with  $O_2$  to 4 atmospheres. Similar results were obtained in experiments on rats, guinea pigs and goats. It is of special interest that this effect of  $CO_2$  should be evident even when the animal was shifted to OHP which was free from  $CO_2$ , a goat which showed no symptoms in a 27 minute exposure to  $O_2$  at 4 atmospheres was subsequently subjected for a few minutes to 22 per cent  $CO_2$  in air at atmospheric pressure after which the chamber was washed out with  $O_2$  and the pressure raised to 3.7 atmospheres. Convulsions then came on in 7 minutes. Hill concluded that "increase of carbon-dioxide tension in the tissues is a factor in the production of convulsions, which follow exposure to high pressures of oxygen." Massart (1934) has likewise observed that in the presence of  $CO_2$  the onset of convulsive seizures was accelerated.

Behnke, Shaw, Shilling, Thompson and Messer (1934) having found that the average  $CO_2$  tension of mixed venous blood of dogs breathing  $O_2$  at a pressure of 4 atmospheres was only 6.5 mm Hg higher and the pH only 0.03 unit lower

(the latter by indirect methods) than that found during exposures to air at 1 atmosphere, thought no significance should be attached to such small  $\text{CO}_2$  changes and stated "It seems highly improbable that the rise in carbon-dioxide tension due to the absence of reduction of the oxyhemoglobin, and the resultant increase in acidity is sufficient to account for the symptoms of oxygen poisoning" In later work, however, Shaw, Behnke and Messer (1934) agreed that "Carbon dioxide tensions which are wholly innocuous when associated with oxygen pressures of less than 1 atmosphere, prove highly toxic when associated with oxygen at 4 atmospheres of pressure", and Behnke and Willmon (1939) state that increased acidity of venous blood (0.03 pH) is "worthy of emphasis" (see below)

Shaw, Behnke and Messer (1934) reported that symptoms of  $\text{O}_2$  poisoning occurred in anesthetized (barbiturate) dogs breathing OHP for prolonged periods, even when alveolar  $\text{CO}_2$  content was maintained at subnormal levels by artificial overventilation, from this it was concluded that  $\text{CO}_2$  could not have been the cause of the reaction to OHP Now artificial overventilation should, of course, tend to lower tissue  $\text{CO}_2$ , but this, it would appear, does not justify their conclusion which is based on the erroneous assumption that tissue  $\text{CO}_2$  and alveolar  $\text{CO}_2$  always run parallel courses Obviously any breakdown in the system which transports metabolites from the tissues to the lungs should result, other things remaining constant, not only in an accumulation of those metabolites at their sites of origin in the tissues, but also, and consequently, in a diminution in their concentration at the pulmonary terminal of the transport system Indeed, barring the factors of compressional inflow and increased diffusion resistance, one would expect to find a subnormal  $\text{CO}_2$  content in the alveolar air of animals exposed to OHP even without artificial overventilation for several reasons—viz, an inadequate carriage of  $\text{CO}_2$  from the tissues to the lungs, slowed diffusion through damaged alveolar membranes, the blowing off of alveolar  $\text{CO}_2$  by the hyperpnea which commonly occurs in  $\text{O}_2$  poisoning, and a diminished tissue metabolism

These authors (Shaw, Behnke and Messer, 1934) do, however, recognize  $\text{CO}_2$  as a contributing cause in oxygen poisoning but believe that its influence "is to render the oxygen more toxic or the tissues more sensitive to the effects of oxygen" On the other hand, Behnke, Johnson, Poppen and Motley (1935) suggest that exposure to  $\text{O}_2$  at atmospheric pressure increases the sensitivity of nervous tissue to  $\text{CO}_2$ , as had Yamada (1918)

Massart (1934) has maintained that the augmentation of response to OHP which occurs on the addition of small amounts of  $\text{CO}_2$  to the respired gas is caused by an increased uptake of  $\text{O}_2$  consequent upon the hypercapnic hyperpnea Anything which increases breathing tends, he says, to hasten the onset of convulsions whereas a decrease in ventilation postpones the onset The less pronounced elevation in blood acidity in those animals which were markedly hyperpneic (Bean, 1931) point to an opposite conclusion In order further to evaluate hyperpnea as a possible contributor to  $\text{O}_2$  poisoning, Bohr and Bean (1942a) subjected tracheotomized, urethanized or decerebrate rats to controlled artificial respiration in  $\text{O}_2$  at pressures of 6 atmospheres, the results of such experiments showed, contrary to Massart's contention, that artificial hyperventila-

tion postponed, rather than hastened the onset of reaction to OHP. This postponement of the onset of the toxic action of OHP might very well be explained on the basis of an improved  $\text{CO}_2$  diffusion gradient from the plasma to the alveolar gas as a result of the hyperventilation. These experimental data point again to the importance of  $\text{CO}_2$  and its disturbed transport in poisoning by OHP.

It is conceivable that  $\text{CO}_2$ , whether it be of exogenous or endogenous origin, might accelerate the onset and augment the reaction to OHP by virtue of its recognized vasodilating action, since an increased blood flow should accelerate the  $\text{O}_2$  saturation of the tissues. On the other hand, vasodilatation with a consequent increase in blood flow should, as already pointed out, tend to maintain the removal of endogenous  $\text{CO}_2$  and so partially compensate for the disturbed  $\text{CO}_2$  transport by hemoglobin, vasodilatation might then postpone or alleviate the response to OHP. But in any case  $\text{CO}_2$  must certainly play a more important rôle than simply one of vasodilatation.

Stadie, Riggs and Haugaard (1944) in reviewing the subject state "there is clear evidence that the *partial pressure* of carbon dioxide in the tissues is *increased* by high oxygen," but maintain that this increase is not due to a retention of  $\text{CO}_2$ , such as might occur, for example, in certain types of pulmonary pathology. They are of the opinion that the failure in the reduction of oxyhemoglobin provides "sufficient explanation" for the increased  $\text{CO}_2$  partial pressure of the tissues.

A careful theoretical analysis by Stadie, Riggs and Haugaard (1944) of the possible rôle which oxyhemoglobin and its non reduction might play in oxygen poisoning, led them to conclude that "the changes in the blood expected on physico-chemical grounds do occur when oxygen excess is inhaled." On the other hand they cite the failure of Behnke et al (1934) to find an increase in the arterial  $\text{CO}_2$  tension, as proof that there is no interference with the transport of  $\text{CO}_2$ . But, provided  $\text{CO}_2$  elimination at pulmonary membranes and ventilation are not diminished, why should one select an increased arterial  $\text{CO}_2$  tension as an infallible index of a disturbance in the transport of  $\text{CO}_2$  from the tissues to the lungs? The hyperpnea which commonly occurs in OHP might lead one to expect a decrease in arterial  $\text{CO}_2$  tension, rather than an increase. To use a rough analogy, the emptiness of trucks on their return trip is not proof of the adequacy of a transport system.

If there is no "retention" of  $\text{CO}_2$ , and if there is no disturbed transport of  $\text{CO}_2$  from the tissues, and if further, as is agreed, there is no increase in the production of  $\text{CO}_2$  in the tissues, how is the increased tension of  $\text{CO}_2$  in the tissues, for which there is "clear evidence," to be explained? Stadie and associates accept the 1934 interpretation of Behnke et al that the non reduction of oxyhemoglobin and the increase in  $\text{CO}_2$  tension, play no significant part in the poisoning by OHP—an interpretation which as indicated above, was later modified (Shaw, Behnke and Messer, 1934) to include  $\text{CO}_2$  as a contributing cause of  $\text{O}_2$  poisoning. This acceptance was based for the most part on three arguments, one of which was, that "the symptoms of  $\text{CO}_2$  acidosis do not resemble remotely those of oxygen poisoning." But why should one expect that the response to  $\text{CO}_2$  in association with OHP to be the same as that of  $\text{CO}_2$  at atmospheric pressure?

Certainly the experimental conditions upon which the  $\text{CO}_2$  is superimposed in each of these two situations are not comparable

If it be assumed that  $\text{CO}_2$  is not causally involved in the reactions to OHP, and if as stated, the reactions to OHP are so remotely different from those induced by  $\text{CO}_2$ , we should expect that in the administration of  $\text{CO}_2$  to animals exposed to OHP, some of the distinctive  $\text{CO}_2$  effects would retain their identity in the animals' reaction to such conditions. But as a matter of fact, all reports indicate that the addition of  $\text{CO}_2$  results, not in any new type of response, but rather, in simply an accelerated precipitation and enhancement of the same responses which are typical of OHP in the absence of the exogenous  $\text{CO}_2$ . The similarity of the OHP and  $\text{CO}_2$  effects is attested to by the statement of Shaw, Behnke and Messer (1934) that the rôle which  $\text{CO}_2$  plays "is to render the oxygen more toxic or the tissues more sensitive to the effects of oxygen."

A second reason for dismissing the non-reduction of oxyhemoglobin and the increased  $\text{CO}_2$  tension, was that "the changes observed were slight." But it should be pointed out that those same changes which were originally considered slight and of no consequence have more recently been re-evaluated in the light of new experiments and are now spoken of as "worthy of emphasis" (see below).

The third argument for dismissing the factors of disturbed transport and increased tension of  $\text{CO}_2$  was the generally recognized fact that the maximal response to OHP does not occur at that  $\text{O}_2$  pressure which is just sufficient to maintain the hemoglobin in a constant state of  $\text{O}_2$  saturation, much more pronounced effects are induced at higher  $\text{O}_2$  pressures. This is of course one line of evidence that there are other factors than the non-reduction of oxyhemoglobin involved in poisoning by OHP but it does not, in the opinion of the reviewer, rule out the increased  $\text{CO}_2$  tension arising from such non-reduction as one of the causative agents.

The argument that hemoglobin involvement in poisoning by OHP is of no consequence because some organisms devoid of this pigment are more readily killed by OHP than are those whose life is dependent upon it, would appear to be invalid for there are on the other hand numerous organisms not possessed of hemoglobin which are strikingly resistant to the toxic action of OHP, the resistance of *Drosophila* (Williams and Beecher, 1944) for example is much greater than that of mice, and this might suggest that hemoglobin is after all an important contributor to the greater susceptibility of higher forms to the toxic action of OHP.

In summary then it may be said that the evidence clearly shows that in exposures to OHP there is an increase in the  $\text{CO}_2$  tension of the tissues, that there is a non reduction of the oxyhemoglobin and that this, in the absence of other changes, provides sufficient explanation for the increased tissue  $\text{CO}_2$  tension. The denial of the importance of the increased  $\text{CO}_2$  tension as a causative factor in poisoning by OHP, based chiefly on the early report of some investigators that this increase was too slight (a view later modified in light of later experiments) appears to be untenable because of the now well accepted fact that an increase in  $\text{CO}_2$ , of otherwise inconsequential magnitude, becomes highly potent in the

presence of OHP This increased potency is manifest by a pronounced enhancement of those reactions typical of OHP in the absence of exogenous  $\text{CO}_2$ , an expectation that  $\text{CO}_2$  in association with OHP would elicit the same response as  $\text{CO}_2$  acidosis at atmospheric pressure—an argument used in the denial of the importance of  $\text{CO}_2$  in OHP—appears to be unwarranted. Where, as has frequently been the case in experiments purporting to show the effects of OHP alone, there is any appreciable concentration of  $\text{CO}_2$  in the respired OHP, it markedly augments the effects of OHP chiefly by throwing additional load on an already disrupted  $\text{CO}_2$  transport system The presence of severe and extensive pulmonary damage which is sometimes induced by OHP in the more prolonged exposures must also contribute to an increased tissue  $\text{CO}_2$  tension and an enhancement of the reactions Taken as a whole the evidence would appear to fully justify the conclusion that the increased  $\text{CO}_2$  tension constitutes an important etiological factor in poisoning by OHP On the other hand  $\text{CO}_2$  and its disturbed transport by the blood cannot be elected as the only etiological factor

4. *Increased Tissue Acidity as an Etiological Factor* An increase in  $\text{CO}_2$  tension in the tissues implies an increase in tissue acidity and, barring some peculiar specific action of  $\text{CO}_2$ , it is fair to assume that the action of  $\text{CO}_2$  in OHP is attributable to its acid properties. Stadle, Riggs and Haugaard (1944) point out that as a result of failure in the reduction of oxyhemoglobin in OHP one should expect an increased acidity of venous blood That an increased blood acidity does occur under these conditions was demonstrated in experiments mentioned above (Bean, 1929, 1931) This increase may be safely accepted as reflecting a shift of tissue pH to the acid direction The findings of Campbell (1929) also indicate the tissue acidity increased in exposures to OHP Bert (1878) speculatively remarked that tissue acidity might be increased in poisoning by OHP and be the cause of death.

Behnke, Shaw, Shilling, Thomson and Messer (1934), while granting that an increase in acidity of venous blood is possible (they found by indirect methods, a change in pH from 7.33 to 7.30) when oxyhemoglobin is not reduced, concluded that this increase is so small as to be insignificant as an etiological factor in  $\text{O}_2$  poisoning Behnke, Johnson, Poppen and Motley (1935) on the basis of experimental data from human subjects state that "Increased tissue acidity resulting from exposure to oxygen is ruled out by the constancy of the respiratory minute volume at 1, 2, 3 and 4 atmospheres' pressure While there are minor fluctuations in minute volume, there is no consistent tendency toward an increase as the oxygen pressure is raised "

Other things remaining constant, an increase in ventilation at atmospheric pressure does suggest increased tissue acidity, on the other hand it would seem unwise to assume that respiratory minute volume by itself constitutes an infallible index of the degree of tissue acidity, particularly in exposures to OHP Certainly hyperpnea and dyspnea are common characteristics of the response of anesthetized (urethane) animals to OHP, yet prolonged apneas have occasionally been recorded in such exposures (Bean, 1932)

The lack of an exact parallelism between the degree of hyperpnea and the

acidity of the tissues in OHP is indicated by the finding (Bean, 1931) that the greatest increase in blood acidity very commonly occurred in those animals whose breathing was least increased by the OHP. This emphasizes the importance of the hyperpnea in OHP as a compensatory reaction against an increasing tissue and blood acidity.

The fact that increased  $\text{CO}_2$  does not under all circumstances or in all concentrations result in increased breathing attests to the inadvisability of assuming that an increased  $\text{CO}_2$  tension and the consequent increase in tissue acidity invariably elicits hyperpnea. Marshall and Rosenfeld (1936) and Moyer and Beecher (1941, 1942 a, b) found that the administration of  $\text{O}_2$  at atmospheric pressure to anesthetized (barbiturates) animals subjected to increased  $\text{CO}_2$  tensions, resulted in diminished breathing and even apnea. These findings provide evidence of both the fallacy and the danger of assuming that increased  $\text{CO}_2$  tension and the accompanying elevation of tissue acidity always increases breathing. The decreased breathing and occasional apnea which not infrequently occur in the late stages of exposures to OHP may be due, in part at least, to a somewhat comparable reaction of the organism to increased  $\text{CO}_2$ . Similarly, the less pronounced response to OHP, of animals decerebrated under evipal anesthesia (Bean and Rottschäfer, 1938) and the relatively low percentage of animals anesthetized with barbiturates which were convulsed by OHP (Behnke et al., 1934), may find partial explanation in this action of increased  $\text{CO}_2$  tensions in the tissues.

Bert (1878) reported that the presence of  $\text{CO}_2$  in the respiratory medium diminished the occurrence of  $\text{O}_2$  convulsions even though the severity of the exposures might be such as to cause death. He attributed this absence of any marked response of some subjects (birds) to OHP, to the  $\text{CO}_2$  which acted on the tissues as an anesthetic to prevent the seizures in a manner similar to that of chloroform. It should be emphasized, however, that because under some circumstances increased  $\text{CO}_2$  diminishes breathing, it does not necessarily follow that excess  $\text{CO}_2$  must have attained such proportions as to depress the underlying vital phenomena, it may very well be that distinctly lesser concentrations of  $\text{CO}_2$  might diminish breathing or hold it temporarily in abeyance, not because of a depressant influence but rather simply because of an interference with, or maladjustment of, the normal integrative mechanisms. The respiratory records presented by Behnke et al. (1935) do not include any tracing of the respiratory minute volume obtaining under exposure to OHP but those tracings which are shown of the breathing in  $\text{O}_2$  at atmospheric pressure do illustrate distinct respiratory changes. The authors suggest that these changes were caused by an increased sensitivity to  $\text{CO}_2$ , induced by the increased  $\text{O}_2$  tension.

The conclusion of Shaw, Behnke and Messer (1934) agrees that even a relatively slight increase in  $\text{CO}_2$  tension becomes peculiarly effective in the presence of OHP and this implies a similarly enhanced significance to the attendant small increase in acidity. Incidentally the results of these experiments illustrate how misleading it is to assume, that because a certain chemical change in an animal appears to be "insignificant" under conditions of normal pressure, it should re-

retain that same insignificance under the vastly different adjustments obtaining in an animal exposed to OHP

The acceptance of increased acidity as a significant contributor in the reactions to OHP is indicated in the report of Behnke and Willmon (1939) on their investigation of the use of OHP in deep-sea diving. In contrast with the conclusion of Behnke et al (1934) on the insignificance of a pH change of from 7.33 to 7.30 these authors state that one of the factors "worthy of emphasis" is, "that the acidity of the venous blood is increased by a pH change of 0.03 when oxygen is inhaled". On the whole, then, the experimental findings and interpretations of earlier workers regarding the significance of  $\text{CO}_2$  and increased acidity in poisoning by OHP find support and confirmation in the later work of other authors who, having originally denied the importance of these factors, have later come to recognize their potency.

One of the various possible modes by which increased acidity might conceivably contribute to the reactions of poisoning by OHP which deserves mention is the effect which increased pH has on synaptic physiology. Gesell, Brassfield and Hamilton (1941, 1942) have presented evidence that one of the determinants of the intensity and duration of cell activity is the pH at the site where cell activation is normally accomplished by the physiologically liberated acetylcholine. Increased OH serves to protect this humoral substance from destruction, thus its duration and intensity of action are increased. One might therefore suspect that an increased tissue acidity arising from the various conditions obtaining in OHP would contribute to the general reaction of the organism by virtue of its influence on the longevity of acetylcholine.

Questioning whether the response of isolated smooth muscle to OHP might not be due to increased concentration of acetylcholine liberated from intrinsic nerve endings Bean and Bohr (1940) found that atropinization failed to prevent the typical response of the tissue to OHP and inferred therefrom that the effect of OHP on isolated smooth muscle was not due to acetylcholine. On the other hand the same authors observed (1940, unpublished data) that OHP does not eliminate the action of acetylcholine artificially administered to tissue under pressure. While these results suggest that the potentiation of acetylcholine by increased acidity does not play an essential rôle in the  $\text{O}_2$  poisoning of isolated tissue preparations, they do not rule out the possibility of its involvement in  $\text{O}_2$  poisoning in the intact animal. The problem calls for further experimental work. In this connection the fact that the normal destruction of acetylcholine is accomplished through the enzyme cholinesterase is of especial interest for if OHP inhibits cholinesterase as it does some other enzymes, acetylcholine effects should thereby be augmented.

The probability that  $\text{CO}_2$  enhances the toxic influence of OHP by virtue of its acid properties, demands a consideration of other mechanisms operative in OHP which may lead to an increased tissue acidity, for example, the decreased metabolism which occurs under such conditions.

*5 Decreased Metabolism as an Etiological Factor* In considering changes in metabolism, cognizance must be taken of the fact that many reports to the effect



that OHP causes a decrease in metabolism have been based upon data taken from experimental animals in those terminal states where such decrease would be expected as a part of the slowing up of all vital phenomena preceding actual death, and do not, therefore, represent any action peculiar to OHP. However, even after necessarily discounting such data there remains convincing evidence that OHP does have a depressant action on metabolism.

The significance attached to the decrease in metabolism induced by exposures to OHP has been variously interpreted. Campbell (1937a, b) has maintained that the decrease in body temperature of animals exposed to OHP, is a protective metabolic reaction against the toxic influence of OHP. Ozorio de Almeida (1934) believed that the decreased metabolism in OHP is the cause of the pulmonary damage and convulsions induced by OHP. Bert held that it represented the actual death of the cells. This decreased metabolism may conceivably represent an ultimate etiological factor in the poisoning by OHP, or it may on the other hand contribute to the reaction by augmenting the tissue acidity. According to Bert the oxidations in animals possessed of hemoglobin failed because the  $O_2$  was not derived from the oxyhemoglobin, the implication being that  $O_2$  so derived had some peculiar property essential to oxidations not possessed by the  $O_2$  which, under OHP comes directly from simple solution in the blood and tissue fluids without intermediary association with hemoglobin. Nevertheless in those forms of life devoid of hemoglobin it was maintained that the excess of dissolved  $O_2$  alone decreased the oxidations. It was further claimed that the decrease in oxidations induced by OHP, unlike that resulting from exposure to low  $O_2$ , was caused by some toxic substance which, produced by the OHP, persisted in the body after the animal's return to normal environment and resulted in ultimate death.

There are however several conditions obtaining in animals exposed to OHP which suffice to explain this decreased metabolism without the necessity of resorting to the introduction of a hypothetical toxic substance. Among such conditions are the increased  $CO_2$  tension and tissue acidity, both of which depress tissue oxidations, another is a direct action of OHP itself on the intimate mechanisms of cellular respiration.

6 *Direct Action of Oxygen at High Pressure, Enzyme Involvement* That OHP has some more direct action on tissues other than that effected through increased  $CO_2$  tension and its disturbed transport by the blood, is demonstrated by the fact that it adversely affects isolated tissue preparations, and kills or injures organisms which possess neither circulatory system nor hemoglobin, and since increased  $O_2$  tension, or pure  $O_2$ , even at atmospheric pressure inhibits many types of enzyme activity (see section I) it would appear that one important avenue through which a direct action of OHP might be accomplished, would be an attack upon cellular enzyme mechanisms. The early experiments of Bert provide suggestive evidence that OHP does impair enzyme activity, he pointed out that the more intimate vital phenomena were affected. But his own general conclusions regarding the effects of OHP on ferments, if correct, would appear to rule out the possibility that enzymes were either directly or indirectly affected.

by OHP According to Bert's designation, enzymes as we now define them would be classed with his "false" ferments—non formed ("ferments non figures") soluble products or extracts of cells—and these he claimed, remained unaltered by OHP It was only the "true" ferments—living microscopic organisms—that were adversely affected, as he says, "les organismes microscopiques qui constituent les vrais ferments sont tués par l'oxygène, qu'au contraire, les ferments non figurés, solubles, les diastases, lui résistent parfaitement et sont même conservés par lui"

Bert observed that although the putrefaction of meat was suspended by its exposure to OHP, it turned acid in reaction, no tests were made but he suggested this might be due to an accumulation of lactic acid as a result of the suspension of the fermentation processes by the OHP Unaware of these early speculations of Bert, Bean and Haldi (1932) posed the question of whether the decreased metabolism induced in animals by OHP might not be reflected in an alteration in the lactic acid of the blood Finding a reversible increase in the blood lactic acid of urethanized dogs exposed to OHP these investigators suggested in explanation, that the toxic effects of OHP involved a poisoning of enzymes with a resultant disturbance in oxidative processes

The experiments of Meyer (1927) provide direct evidence that OHP alters enzyme activity, that author found that suspensions from macerated brain tissue which had previously been exposed to  $O_2$  at pressures slightly less than 4 atmospheres for 4 hours, were less efficient in oxidizing guaiacum in the presence of hydrogen peroxide than those of similar tissue preparations which had been exposed to atmospheric air for an equal period It was concluded that OHP caused a decrease in metabolism of the brain

Libbrecht and Massart (1935) found that in the blood of rabbits which had been convulsed by exposures to  $O_2$  at 4 atmospheres for 30 minutes, the ratio of the oxidized to reduced glutathione was decreased to one-tenth of its normal value and believed this was causally related to the impaired oxidation in the tissues which obtains in such exposures Libbrecht and Massart (1937) using a chamber which provided means of controlling the necessary experimental procedures from outside, studied the effects of OHP on the preparations in question while they were still subjected to the increased pressure They found that the exposure of fresh succino-dehydrogenase preparations to  $O_2$  at between 4 and 5 atmospheres poisoned the system so that the  $O_2$  consumption was completely stopped But in experiments in which the activity of the cytochrome system in the preparation had previously been eliminated by the use of cyanide, this poisoning of the dehydrogenase system was absent The authors concluded from this that molecular  $O_2$  is not toxic but that it becomes so when activated by the cytochrome system, according to them it is active  $O_2$  which inhibits the dehydrogenase system

Stadie, Riggs and Haugaard (1944) state that the term "l'oxygène actif" of Libbrecht and Massart is not sufficiently specific and suggest that perhaps the hypothesis of these authors might be re-framed by stating that the cytochrome system together with the oxygen at high pressures oxidizes the succino-dehy-

drogenase to an inactive form " But in their own experiments Stadie and associates found no evidence to support this hypothesis

Yet the experiments on rabbit intestine (Bean and Bohr, 1940, unpublished data) indicate that the cytochrome, or some other cyanide-sensitive system does play a rôle in the toxic action of  $O_2$  at high pressure on isolated tissue for it was found that although  $O_2$  pressures of from 3 to 6 atmospheres caused a rapid drop in the tonus of isolated pyloric sphincter, this depressant action of high  $O_2$  pressure could be eliminated by previously treating the tissue with NaCN which of itself caused no change in tonus The fact that NaCN caused no appreciable change in tonus of the pyloric sphincter was interpreted to mean that the maintenance of tonus in that tissue is not dependent upon a cyanide-sensitive system and since in the absence of NaCN,  $O_2$  at high pressure causes a sharp drop of tonus it would appear that  $O_2$  at high pressure affects enzyme systems other than those which are cyanide-sensitive The results suggested further that the enzyme systems responsible for the maintenance of tonus in the pyloric sphincter and in the circular duodenal muscle are not identical

Bohr and Bean (1940) in experiments on fresh extracts of pork hearts found that exposure to  $O_2$  at pressures of 7.5 atmospheres, irreversibly decreased succino-dehydrogenase activity by as much as from 9 to 50 per cent, and suggested that the incomplete reversal of the toxic effects of OHP on isolated tissues previously noted, might be explained by such an irreversible inactivation of enzyme function These authors proposed that considerable variation in the degree of inactivation of the enzyme preparations by OHP might explain the striking individual variation in the susceptibility of animals to the toxic effects of OHP so frequently observed The greater resistance of young animals to the toxic action of OHP, as reported by some investigators, the enhancement of resistance by thyroidectomy (Campbell, 1937b) and by starvation (Ozorio de Almeida, 1934) might also be explained by a certain enzyme state peculiar to each of those conditions The great variety of susceptible enzyme systems may further contribute to the wide variation in the susceptibility to the toxic action of OHP seen not only in different individuals but also in different animal forms

By far the greater part of the evidence of enzyme inactivation by  $O_2$ , which at best is limited, has been derived from experiments carried out with increased tensions of  $O_2$ , or pure  $O_2$ , at atmospheric pressure and it is not unlikely that some of this evidence is applicable also to the effects of  $O_2$  at pressures of several atmospheres But in assuming that the same effects, somewhat enhanced, should necessarily occur in OHP as in  $O_2$  at atmospheric pressure some caution may be demanded The case of hemochromogen enzymes may perhaps be illustrative of this Hoberman and Rittenberg (1943) found that hydrogenase along with hydrogenlyase, enzymes first described by Stephenson and Stickland (1931), is reversibly inactivated by oxygenation for 24 hours This inactivation was attributed to an oxidation of a heavy metal portion of the enzyme which they believed was an iron porphyrin-protein complex They concluded that in the presence of  $O_2$  the enzyme is in the oxidized state but in the presence of hydrogen and reducing agents it is in the reduced state According to this, then,

the toxic action of  $O_2$  on the enzyme, previously noted by several investigators (Stephenson and Stuckland, 1931, Wilson, Lee and Wilson, 1942), might be explained simply as the transformation of the iron to the oxidized state, in which form the enzyme is inactive, whereas reduction of the iron constitutes a reactivation of the enzyme and so reverses the toxic action of the  $O_2$ .

Stadie and associates (1944) accept this as one of the well substantiated theories of enzyme inactivation by  $O_2$  but their own experiments showed that catalase, which is another hemochromogen comparable to that of hydrogenase, was not inactivated by  $O_2$  even at high pressures. The authors point out that this indicates that all iron or metallo-hemochromogen enzymes may not react in the same way to  $O_2$ . However, perhaps one may question whether these results may not also indicate that hemochromogens react differently in  $O_2$  at high pressure than they do in increased  $O_2$  tensions at atmospheric pressure. Hoberman and Rittenberg found that increased pressure of heavy hydrogen in their experiments (470 mm Hg) decreased the activity as compared with that at a pressure of 30 mm Hg.

Another reason for questioning the expectation that the effects of  $O_2$  on enzymes at high pressure should be the same as those at atmospheric pressure is the fact that catalytic action in some purely physico-chemical systems has been found to be distinctly altered by increased pressure. One explanation offered for this is that increased pressure induces a molecular rearrangement at interfaces. There would seem to be no justification for denying the possible occurrence of similarly important molecular rearrangements in biological systems.

In spite of the probable differences in the effects of  $O_2$  at atmospheric and at high pressures, the literature concerning enzyme inactivation by  $O_2$  at atmospheric pressure offers a variety of suggestive possibilities by which OHP may inhibit enzyme activity. Stadie and associates (1944) group those they consider most significant as follows: (1) oxidation of a co-enzyme to the inactive oxidized form, (2) oxidizing activating sulfhydryl compound, (3) oxidizing active —SH groups of the enzyme molecule, (4) oxidation of metallo-hemochromogens to inactive oxidized forms, (5) oxidizing the activating metal constituent, (6) formation of an inhibitor from precursor other than the enzyme, (7) alterations in the oxidation-reduction potential of the medium.

Fahr (1941) reported that intra peritoneal injection of lactoflavin, nicotinic acid amid ("Nicobion" Merk) decreased the toxic action of OHP (4 to 6 atm) in subsequent exposures. Glutathion likewise was of some, but much less, benefit. These effects were thought to be explainable on the basis that OHP had a toxic action on a redox system in the enzyme mechanisms.

The activity of most enzyme systems is sensitive to changes in the reaction of their immediate environment so that even relatively small shifts in pH become very significant. This is illustrated by the pH curves of Wilson, Lee and Wilson (1942) which show that a sharp decrease in the activity of the enzyme hydrogenase occurs (at atmospheric pressure) when the pH falls only slightly below the optimum of about 7.3. The striking enhancement of the reaction to OHP, both in animals possessed of hemoglobin and in those such as *Drosophila* which

are devoid of this pigment, may find an explanation then in the acid effect of  $\text{CO}_2$  in combination with the influence of OHP itself, on tissue acidity. This would appear to be particularly significant since the optimum pH of many enzymes other than hydrogenase are in the neighborhood of 7.4 (Koehler and Reitzel, 1925, Lehmann, 1935, McGowran and Rheinberg, 1933).

In summary then it may be said that the evidence from experiments on isolated tissues, and extracts therefrom, together with data on enzymes from other sources, indicate that fundamental cellular metabolism can be altered by a direct action of OHP on enzymatic processes. This direct action, operative in the absence of a circulatory system, is not dependent upon a disturbed  $\text{CO}_2$  transport function of hemoglobin.

The fact that  $\text{CO}_2$  augments the toxic effects of OHP on organisms devoid of hemoglobin such as *Drosophila* and some bacteria must mean that  $\text{CO}_2$  has some distinct adverse effect of its own, perhaps due to its acid properties, when associated with OHP or that it potentiates the direct toxic action of the OHP on enzyme systems.

In those animals possessed of hemoglobin the direct effect of OHP on tissue enzymes must share a goodly portion of the responsibility for the reactions and decreased metabolism which exposure to OHP induces. But in these animals there is, in addition to this direct effect of OHP by itself, the increased tissue  $\text{CO}_2$  tension and tissue acidity consequent upon a disturbed  $\text{CO}_2$  transport. This  $\text{CO}_2$  acting in the presence of OHP, perhaps in a manner similar to that suggested above in connection with response of animals devoid of hemoglobin, augments the reactions of the animals to OHP.

If, further, there be  $\text{CO}_2$  present in the environmental OHP or if  $\text{CO}_2$  elimination at the lungs is impaired, there is thrown an additional load on an already disrupted  $\text{CO}_2$  transport system and there results a more rapid onset and a still further augmentation of the intensity of the OHP reaction. The direct toxic action of OHP on respiratory enzymes, the influence of increased  $\text{CO}_2$  tension either by itself or in some potentiative combination with OHP on enzyme mechanisms, and the acid effect of  $\text{CO}_2$ , all tend toward an increased tissue acidity and decreased metabolism. In OHP, the situation would seem to be such that not only does increased acidity, such as that arising from  $\text{CO}_2$ , disrupt enzyme activity, but also that OHP, by directly disrupting enzyme activity, further increases tissue acidity.

**7 Hyperoxic Anoxia** The reactions of isolated longitudinal muscle of rabbit duodenum, to OHP, to sodium cyanide, and to low  $\text{O}_2$  have been found (Bean and Bohr, 1940) to be so very similar as to suggest that a common factor is operating in all three conditions, this might very well be an increased tissue acidity, which in the case of both the cyanide and the OHP may arise from the diminished  $\text{O}_2$  utilization consequent upon a poisoning of respiratory enzymes. Or it might be that the low  $\text{O}_2$  utilization, which amounts to a hypo- or an anoxia, is itself the more fundamental factor common to these three conditions. This seemingly paradoxical relationship where, in OHP, the superabundance of  $\text{O}_2$  induces tissue changes and elicits responses, which are typical of those induced by  $\text{O}_2$  deficiency, has been referred to as "hyperoxic anoxia."

There are numerous other observations, especially those from intact animals which lend support to the view that in both anoxia, and in poisoning by OHP, the same fundamental processes are affected. For example, it may be more than coincidental that the nausea, vomiting, defecation, pupillary dilatation, muscular tremors, prostration and convulsions seen as symptoms of cyanide poisoning, of asphyxia and of the anoxia of altitude or mountain sickness, are also found in poisoning by OHP. Ravenhill (1913) found that convulsive seizures occurred in altitude sickness, Kaunitz (1942) observed anoxemic convulsions in mice and convulsions occur not infrequently in routine testing of aviation cadets in low pressure chambers (Gemmell, 1942).

Haldane and Priestley (1935) pointed out that in diving operations where the  $O_2$  of the respired gas was about 2 atmospheres, the reactions of the workmen, particularly the loss of memory and abnormal behavior, "were strongly suggestive of an effect on the brain similar to that caused by too low an oxygen-pressure." The several reports to the effect that young animals are more resistant to the toxic action of OHP than older ones constitutes still another point of similarity between  $O_2$  poisoning and anoxia, since it is found that young mice are more resistant to cyanide and to asphyxia than old mice (Reiss and Haurowitz, 1929).

Further suggestive evidence that the toxic action of OHP may be essentially due to an anoxia is found in the similarity between some of the late responses to OHP in experimental animals (disturbed equilibrium, neuro-muscular incoordination, gait and posture) and those of alcoholic intoxication, the effects of which have been long likened to those of low  $O_2$  (Ravenhill, 1913, Barcroft, 1920, 1925, Haldane and Priestley, 1935) and have been more recently recognized as a form of real anoxia (Palthe, 1926, MacFarland and Barach, 1936, MacFarland and Forbes, 1940).

It is well recognized that anoxia may be attended by striking changes in personality and this too has a counterpart in poisoning by OHP, docile, tame white rats have not infrequently been changed to combative vicious, biting animals by exposure to OHP. Another feature common to both low  $O_2$  and to OHP, is the wide individual variation in susceptibility to the deleterious action of each of these conditions. Finally OHP (Ozorio de Almeida, 1934) like low  $O_2$  (Monge, 1942, 1943) causes sterility in male animals.

Taken as a whole the evidence forms a rather substantial basis for the interpretation that hyperoxic anoxia arising from enzyme poisoning, and intimately bound up with tissue acidity, constitutes an exceedingly important etiological factor in the production of, if it is not the ultimate cause of, the toxic action of OHP, an animal poisoned by OHP is in effect then drowned in  $O_2$ .

8 *Increased Oxidation in the C.N.S.* Finding that thyroidectomy increased the resistance to the toxic effects of OHP, Campbell concluded (1937b) that  $O_2$  poisoning is due to an "excessive and rapid oxidation in the nerve cells, and that the usual end products of metabolism, e.g., carbon dioxide, are responsible for the poisoning." Bounhiol (1929) likewise held that metabolism was increased by OHP and suggested that this, in conjunction with an impaired removal of metabolites, would result in an excessive accumulation of metabolites which

would eventually slow and limit the reaction in conformity with the law of Le Chatelier

Certainly in view of the disruption in the carriage of  $\text{CO}_2$  by the blood as occurs in OHP, and the peculiarly high potency of  $\text{CO}_2$  and OHP in combination on cellular processes, anything which augments metabolism, such as increased thyroid activity or elevated body temperature, should be particularly effective in hastening the onset, and enhancing the severity of the reaction to OHP. To date, however, there appears to be no very reliable indication that OHP does increase metabolism, in fact with few exceptions the evidence, as previously stated, points to the contrary. Campbell (1937a) has himself maintained that the fall in body temperature which, according to a number of investigators takes place in OHP, constitutes a protective reaction against its toxic action. Granting that such a drop in temperature does take place and that it serves a protective function it is difficult to reconcile such effects with the proposed concomitant increase of metabolism within the C N S.

It has been generally accepted that an increase in environmental temperature does augment the toxic action of OHP but in spite of the importance of the temperature factor in  $\text{O}_2$  poisoning, a fall in body temperature is not necessarily a guarantee against the occurrence of the toxic effects, nor is an elevation in temperature a sure index that convulsions will occur. The temperature of Thompson's alligator rose from  $51^\circ$  to  $75^\circ\text{F}$  during an exposure to  $\text{O}_2$  at a pressure of about 4 atmospheres, and under ordinary circumstances it would be fair to assume that there was an accompanying increase in metabolism, yet no convulsive attack occurred. On the other hand his warm-blooded animals, the temperature of which dropped during the exposure, suffered severe  $\text{O}_2$  convulsions. These observations argue against the excess oxidation theory of  $\text{O}_2$  poisoning. But even if this theory were entirely acceptable, the increased oxidation would only be an intermediate step on the road to a more rapid  $\text{CO}_2$  accumulation, to increased tissue acidity, and thus to the eventual disruption of normal oxidative processes.

**9 Toxic Substances** The idea that poisoning by OHP might be due to some toxic substance seems to have originated with Bert, who, as already mentioned, claimed that the reactions to OHP, particularly the convulsive seizures, must be due to some toxin produced by OHP and which primarily affected the C N S and continued to do so even after the animal's return to normal atmospheric pressure. He was unable, however, to reproduce in normal animals either the convulsions or any of the symptoms of  $\text{O}_2$  poisoning by transfusion of blood from those suffering  $\text{O}_2$  poisoning. He concluded therefore that the convulsive seizures were not due to circulating toxins. He did hold, nevertheless, that death from OHP was caused by some toxic substance produced in the tissue by OHP as he says, "Il semble qu'il se soit, sous l'influence de l'oxygène comprimé, formé dans les éléments anatomiques quelque produit toxique, qui ne peut pas toujours s'éliminer, et tue alors même que sa cause formatrice a disparu. Aller plus loin que cette hypothèse me paraîtrait une impudence dans l'état actuel de la science."

The observation of Barsoum and Gaddum (1936) that the blood of patients

suffering from superficial burns from high temperature in the 21 per cent  $O_2$  of the normal atmosphere contained increased amounts of histamine, suggested to Campbell (1937c) that OHP (4 to 6 atmospheres) at 33°C might lead to similar "burning" of some tissue, especially that of the lung, and result in an increase of blood histamine. This view, reminiscent of the early theories regarding the incendiary action of  $O_2$  on the lungs was tested experimentally. It was found that blood histamine of rats exposed to  $O_2$  at 5 atmospheres was about 3 times that of normal rats, yet histamine injected into normal rats failed to produce the symptoms of  $O_2$  poisoning or to augment the symptoms induced by exposure to OHP. Attempts (Campbell, 1937b) were made also to duplicate the picture of  $O_2$  poisoning by injecting into normal rats not only the blood but also extracts of brain and liver from rats previously poisoned by OHP. These injections likewise failed to elicit the response of  $O_2$  poisoning, injections of similar extracts mixed with thyroxin were also without effect. The experimental results of Iwanow, Krawtschinsky, Prikladowsky and Ssonin (1934) likewise failed to prove  $O_2$  poisoning was caused by some special toxic substance, and Bean and Bohr (1940) were unable to demonstrate that any toxic substance was released from isolated smooth muscle poisoned by OHP.

10 *Psychological Factors* In connection with the discussion of the effects of compressed air some stress was laid on the fact that man or other experimental animal under emotional strain is not quite the same chemical or physical machine that he is under ordinary circumstances. This would seem to be applicable also in the exposures to OHP, while a given chemical or neuromuscular adjustment of psychological origin might exist at atmospheric pressure without gross manifestations, the superimposition of OHP may provide a combination which induces reactions which would not be precipitated by each of these conditions alone. The evaluation of such combination of conditions is extremely difficult, if not impossible, but such difficulty provides no excuse for their dismissal as non-existent.

In experiments on rats known to be particularly susceptible to audiogenic convulsions, the reviewer has observed that the noise of the escaping gas, as occurs on decompression from increased pressures, has in several animals been sufficient to precipitate seizures. It is not unlikely therefore that the addition of the audiogenic influence to that of the convulsant action of OHP may, in some instances, help to precipitate reactions to OHP which otherwise might not occur. This suggests the possible importance of an involvement of psychological factors in the precipitation of both the subjective and the objective reactions to OHP. The wide individual variation in the psychological element may provide another explanation for the wide individual variation in the response to OHP which has been so commonly observed in both man and other experimental animals.

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\* In a report which appeared after the type for this review had been set up, Reinhard et al (1944) described effects of  $O_2$  at from 70 to 100 per cent concentration administered at atmospheric pressure by mask over several periods of from 8 to 20 days duration to 4 human sickle cell anemia patients. They found a decrease in the degree of intravascular sickling of red blood cells, no consistent change in the rate of hemolysis a depression of erythrocytogenesis and a post-administrative increase of circulating leucocytes. The toxic manifestations of the  $O_2$  administration were considered as of only minor significance, their case reports include the following, sore throat and fever pains of from mild to severe intensity in the legs and back during and after the  $O_2$  administration, numbness tingling and stiffness of the hands and feet, sharpshooting pains in the chest, intense headaches persisting for a week after the cessation of the  $O_2$  therapy, feeling of exhaustion and profound weakness, anorexia and loss of weight, nausea and prolonged vomiting peculiar taste sensations, impairment of hearing, dizziness, muscle soreness, cough with mucoid sputum hoarseness, burning sensation in nose and throat epistaxis, swelling and oedema of mucous membranes (Reinhard, E H., C V Moore R Dubach and L. Wade. *J Clin Invest* 23: 682 1944 )

# CERTAIN ANIMAL VENOMS AND THEIR PHYSIOLOGIC ACTION

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One of the minor effects of global war has been the stimulation or intensification of interest in the fauna and particularly the poisonous animals found in distant places. Handbooks on a wide variety of subjects have been prepared for the use of our armed forces. One devoted to the poisonous reptiles of the world attests the importance of a knowledge of this group of potentially dangerous animals (1). Information on bees, spiders and scorpions is probably of equal importance for those who must campaign in tropical and semitropical regions. The current use of animal venoms as therapeutic agents lends a practical aspect to a review of this subject at the present time.

In preparing this paper it was hoped that the complete literature published during the last five years might be covered but this rather modest ambition could not be realized. The literature on the subject of poisonous animals is widely scattered and in many instances is written in a language wholly unfamiliar. Occasionally some idea was gained of the contents of certain papers through abstracts and reviews but, when possible, the original paper was consulted. In short the incomplete nature of this review is freely admitted.

**GENERAL CONSIDERATIONS** Poisonous animals are not confined to one or two classes of the animal kingdom but are found in practically every major division from the lowest to the highest, as has been ably demonstrated by Phisalix (2).

In the present paper chief attention has been given to those animals that are acknowledged to be potentially dangerous to man. With few exceptions they occur among the insects, spiders, scorpions and reptiles. Geographically, poisonous animals are more abundant in the tropical and semitropical regions of the earth and the seas that surround them than in the temperate regions. The poisons of most animals are used primarily to obtain food and secondarily for protection. Since man cannot be used by any poisonous animal for food, bites and stings that he receives are to be considered in the nature of accidents.

The material on each group of poisonous animals will be concerned with chemistry, pharmacology, symptomatology, pathology, treatment, immunity and use of the poisons as therapeutic agents.

**COELENTERATES** Certain jellyfish are known to be dangerous to man. The Portuguese man-of-war is able to cause in man alarming and serious symptoms. Two cases of severe poisoning have been recently described by Stuart and Slagle (3). Little is known of the chemical nature of the venom and its pathologic effects have not been studied thoroughly. The symptoms of jellyfish poisoning are well known and have been ably presented (3) as follows: severe burning pain in the area of contact, cramps in the muscles of the trunk and extremities, board-like rigidity of the abdominal muscles, respiratory difficulty with severe mucous congestion and spasm of the muscles of respiration, profuse nasal and bronchial secretion with incessant cough, profuse lacrimation and perspiration. Because

of the striking similarity of the symptoms to those following an effective bite of the black widow spider (*Latrodectus mactans*), calcium gluconate (10 cc of a 10 per cent solution) was given intravenously with prompt relief of the muscle spasm and respiratory difficulty. The authors offered the suggestion that the stings of jellyfish are more dangerous in tropical than in temperate waters.

**ARTHROPODS.** *The honeybee (Apis mellifera)* The current use of the venom of the honeybee as a therapeutic agent has revived interest in its chemistry and physiology.

The response to intradermal, subcutaneous and intravenous injections of the venom of the honeybee is strikingly like that resulting from injections of the venom of the rattlesnakes *Crotalus horridus*, *Crotalus atrox* and so forth (4), and of the cobra (5). The blood pressure of rabbits and dogs usually shows a temporary elevation, which is followed after a few seconds by marked depression. If the dose is sublethal, the blood pressure returns to the control level and is extremely refractory to subsequent larger doses of bee venom (tachyphylaxis). Repeated large doses can be given without causing a serious decrease of blood pressure. If the injections are continued, death of the animal finally results but is preceded by paralysis and convulsions (6). It is of interest to note that Vellard and Huidobro (7) found that repeated doses of the venom of *Crotalus terrificus* and *Bothrops alternata* given to rabbits produced each time less effect until massive doses did not affect the blood pressure. Neither venom protected against the action of the other. Both venoms lost this protective power after being heated to 65° C. Also an abrupt but transient increase in resistance of pigeons to the venom of *Crotalus adamanteus* has been described (8). A satisfactory explanation of these observations awaits further research.

Derevici, Derevici and Gingold (9) found in one series of experiments on rabbits constant leukopenia (about 3,000 cells in each cubic millimeter of blood) and reduction of erythrocytes. They also found decreased concentration of hemoglobin in four cases but increased concentration in three cases following the sting of 100 bees. The animals that survived for two hours were bled and observations made. In another series of rabbits that received five, twenty five, fifty and one hundred stings respectively there was a decrease of 1,000,000 to 2,000,000 erythrocytes per cubic millimeter of blood. This reduction did not appear to be related to the dose. In this experiment there was an increase in the number of leukocytes, which was in contrast to the leukopenia in the other experiments. It has been reported that rabbits stung by eighty five bees each and studied five or six hours later have no change in blood sugar, a moderately increased blood urea, a markedly increased blood cholesterol, a slight increase in blood calcium and a decrease in the blood chlorides. The convulsions resulting from bee venom intoxication were attributed to the decreased blood chlorides. These authors failed to obtain a neutralization of bee venom by serum from guinea pigs made immune to it (10). Kendrick and Essex (6) were unable to protect other dogs with serum of dogs highly immune to bee venom.

Bee venom and cobra venom cause a long lasting output of epinephrine when injected into the celiac artery of cats after evisceration. This finding was

thought to be due to the formation of lysolecithin in the adrenals. Lysolecithin caused a similar output of epinephrine. After repeated large doses of venom or lysolecithin the medullary cells became unresponsive to further stimulation (11). Bee venom and ergotamine abolished the inhibiting action of epinephrine and certain other agents on the isolated perfused intestine (12).

The pathologic effects of acute and chronic bee venom poisoning in mice have been reported (13). The outstanding macroscopic finding at necropsy was gross hyperemia of the lungs, the spleen, the liver and the kidneys. Microscopically the principal finding was an inflammatory reaction of various organs characterized by perivascular infiltration.

Cornil, Paillas and Chouquet (14), working with rats and guinea pigs, paid particular attention to the effect of the venom on the nervous system. With sufficient doses the animals showed paralysis of the limbs that had received the bee venom, torpor, sleepiness and generalized convulsive reactions. Histologically there were degenerative alterations of the nerve cells and demyelination of the fibers of the peripheral nerves. Similar findings were reported as a result of poisoning by scorpions (15).

There is being accumulated a growing body of evidence that the severe reaction of some persons to the sting of bees is owing to hypersensitivity to certain proteins which are not necessarily a part of the poison itself (16, 17, 18, 19, 20, 21, 22). Desensitization has been accomplished by antigens prepared from the whole body of the insect (16). The toxicity of the venom has been reduced by treatment with glutathione and by theolactin (23).

As a therapeutic agent bee venom has been used for chilblains (24), arthritis (25, 26, 27, 28, 29, 30, 31), neuritis (32) and trachoma (33). The benefit that attended its use in certain cases has been attributed 1, to its action as a counter irritant (26), 2, to its vasodilator action resulting in increased metabolism (27), and 3, to stimulation of the nervous system (14). A certain number of arthritic patients appear to be benefited more or less by the use of bee venom therapy but it is questioned whether this method offers enough promise of advantage over other methods of treatment to justify its further trial (29). It is believed by some observers that bee venom is worthy of consideration and further study in the treatment of arthritis (25, 26, 28, 30, 31). Crain (34) as well as Hollander (29) expressed doubt whether it is worth what it costs the patient in money, time and inconvenience.

*Scorpions and scorpion stings.* Myths concerning certain animals have been evolved and still persist in spite of scientifically established facts that deny their truth. Waterman (35) has given an account of the common scorpion of Trinidad (*Tityus trinitatis*) and Sergeant (36) has reported on some erroneous ideas concerning the scorpions of Algeria. It is agreed that scorpions, quite unlike snakes, are highly susceptible to their own venom but the myth that, if surrounded by fire, they commit suicide by stinging themselves has been exploded. An informative review on the subject of scorpion sting has been written by Roch (37).

Of the fifteen species of scorpion in Arizona only two are capable of doing seri-

ous harm to man. They are *Centruroides sculpturatus* and *Centruroides gertschi*. Their venom is neurotoxic, causing rapid death or convulsions and other grave symptoms in rats. The stings of the other species cause only a local reaction, usually not more serious than the sting of a bee or wasp (38).

Kent and Stahnke (39) have stated that since 1919 more deaths have resulted from scorpion sting in Arizona than from the bite or sting of any other venomous animal. They did not state the number of deaths. The toxicity of the venoms of the striped scorpion (*Uroctonus spinigerus*), the giant hairy scorpion (*Hadrurus hirsutus*) and the yellow slender tailed scorpion (*Centruroides sculpturatus*) was tested by causing them to sting rats. Only the sting of *Centruroides sculpturatus* was serious for rats, which succumbed ten to ninety minutes after an effective sting. The local effects were minimal. General symptoms were pronounced. Children are much less resistant to the venom than adults. Unless early and adequate treatment is given, the outcome is usually fatal to infants.

De Magalhães (40) studied experimentally the venom of four of the thirty-eight or thirty nine species of scorpion in Brazil, namely, *Tityus bahiensis*, *Tityus serrulatus*, *Tityus dorsomaculatus* and *Bothriurus* (species?). He stressed the importance of knowing the amount of venom injected into experimental animals and described a method of collecting the venom. In 13 per cent of 985 human cases of scorpion sting the outcome was fatal. In 161 cases studied from this point of view, the sting was inflicted on the lower limbs in 25.71 per cent, on the upper extremities in 57.23 per cent, in other places in 4.76 per cent and in an unknown site in 12.35 per cent.

According to Barros (41), from 1919 to 1936 there were 629 cases of scorpion sting in Belo-Horizonte, Brazil, all of which were due to *Tityus serrulatus*.

Among the twelve species of scorpion found in Palestine Shulov (42) has shown by animal experimentation that only one species (*Buthus quinquestriatus*) is dangerous. The sting of this species may prove fatal to children and serious for adults. In one year this author traced four fatal cases among children between the ages of six and thirteen years.

Sergeant (43) listed the scorpions of North Africa in the order of their toxicity as follows: *Prionurus australis*, which is responsible for most of the fatal cases, *Prionurus houvillei*, whose sting may prove fatal, *Buthus occitanus*, which kills only rarely, and *Heterometrus maurus*, whose sting has never been known to cause death.

There is essential agreement among writers on this subject that the venom of the dangerous species of scorpions is highly neurotoxic. The local reaction is usually painful but in general is of minor consequence. There may be hemolysis, hemorrhages, cytolytic (local sloughing) and other effects but they are of secondary importance since they are seldom lethal in themselves. De Magalhães (44) has shown a close parallelism between the symptoms of syringobulbia and those of severe scorpion poisoning. Alarming symptoms usually appear within two hours after a serious sting. In addition to local symptoms the following general manifestations usually appear: 1 sweating, disorders of temperature regula-

tion, agitation, headache and dizziness, 2, disorders of respiration increases or decreases in respiratory rate, paralysis, coughing, sneezing and profuse nasal secretion, 3, circulatory disturbances tachycardia or bradycardia, extrasystoles, peripheral vasoconstriction, increased blood pressure and precordial pain, 4, digestive symptoms nausea and vomiting, salivation, defecation (voluntary or involuntary) and diarrhea, 5, urinary effects micturition (voluntary or involuntary), hematuria and anuria, and 6, sensory disturbances visual disorders (blindness) and disorders of taste

As this array of symptoms resulting from severe scorpion poisoning is considered one is reminded of the close similarity to the effects of the venom of certain rattlesnakes, which is predominantly peripheral in action. Consequently it seems proper to inquire whether the case for the central action of scorpion venom has been made too strong (15, 44). However, in support of the "central action" thesis Hasson and Mohammed (45) and later Mohammed (46) working with *Buthus quinquestratus*, reported that paralysis of the parasympathetic nervous system by atropine and of the sympathetic nervous system by ergotoxin entirely protected animals against lethal doses of the venom of this species. On the other hand, Mohammed (46) has shown by animal experimentation that the toxins of the Egyptian scorpions act directly on the endings of the autonomic nervous system.

Among the pathologic changes observed (15) in twenty-two rabbits killed by injections of *Tityus serrulatus* venom were generalized hyperemia and vasodilatation of the brain, particularly evident in the medulla and pons. Hematomas were found in the region of the pons and medulla arising from ruptures of the basilar artery in 40.9 per cent of the twenty-two rabbits. Microscopically there were hyperemia of the brain and marked capillary dilatation, with changes in the vegetative centers consisting of cellular swelling, liquefaction and chromatolysis. Silver stain showed pronounced hyperchromasia and swelling of the intracellular neurofibrils of the pons and medulla. Since the cells of the vegetative centers were specifically damaged, Barros (15) expressed the belief that the venom has an elective effect on these centers and that the symptoms of scorpion poisoning support this concept.

Hasson and Mohammed (45) and later Mohammed (46) reported that proper doses of atropine alone or ergotoxin alone or these two drugs in combination protect rats and dogs against as much as two lethal doses of scorpion poison. Compresses of ammonium hydroxide may be of benefit if applied within ten minutes after the sting (39). Good results are claimed for application of cold to the site of the sting (38). Rao (47) claimed that sodium bicarbonate neutralizes scorpion poison both in vitro and in vivo. All workers are agreed on the advisability of giving scorpion venom antiserum whenever possible in serious cases. In the opinion of most authorities administration of antiserum is the only measure that offers any hope for the victims of severe scorpion sting and then only if adequate doses are given early. This treatment has reduced the mortality rate from 42 per cent to about 18 per cent (40). Sergeant (36) reported that in forty-nine (81 per cent) of sixty cases in which there were alarming symptoms the patients were saved by the use of antiserum. Other treatments are symptomatic. Morphine

is said to be dangerous but barbiturates are thought to be less so for cases of scorpion poisoning. Bromides in large doses are recommended as being of value (39). Basu (48) recommended application of ammonia to the site of the sting and injection of 2 per cent solution of procaine hydrochloride with epinephrine around the puncture to ease pain. As supportive measures saline solution and solution of glucose were given rectally or subcutaneously and camphor in ether as well as epinephrine was given intramuscularly. For the pulmonary edema, atropine was administered subcutaneously. Calcium was also administered intramuscularly. Of nineteen persons who had been stung by scorpions twelve were cured, five died, one was relieved and the other was "discharged otherwise."

Antiserum for scorpion sting has been produced in horses at the Pasteur Institute, in Brazil cattle are used (40). To obtain enough venom to immunize large animals is a heroic undertaking, since as many as 20,000 scorpions are necessary for complete immunization of a single cow (40). Rabbits, dogs, goats (46) and other small animals have been used. Mohammed (46) recommended the use of 0.33 mgm of atropine per kilogram of body weight and 0.005 mgm of ergotoxin per kilogram in the early period of immunization to protect the animal against the lethal effect of the venom. When the animals had become immune to several lethal doses of the venom the "pharmacologic antagonists" were dispensed with. A technic has been described for the preparation of amorphous and crystalline scorpion toxin which appears to be more potent than that produced by previous methods (49). A toxoid or anavenin has been prepared (50, 42) by treating the venom with formalin. This procedure has been of great aid in the rapid production of antiserum for scorpion poison. The highly specific nature of the antiserum of certain species is emphasized by Varela (51), who found that horse serum highly immune to the venom of *Centruroides limpidus limpidus* did not neutralize the venom of *Centruroides suffusus*. Failure to produce an active toxoid by treating the venom of *Centruroides limpidus limpidus* has been reported. Fish are more susceptible than frogs to the venom of *Centruroides limpidus limpidus* and cats and pigeons are very susceptible.

Many cases are on record of dramatic recovery from serious symptoms following the use of scorpion venom antiserum (52, 39). Severe crippling, supposedly the result of scorpion sting, has been described (52). Scorpion venom, so far as I have been able to find, has not been used in the treatment of any human ailment in modern times.

Our knowledge of the chemical nature of the venom of scorpions is practically nil.

**Spiders** Spiders, like the snakes, in respect to the action of their venom, may be divided roughly into two groups. Those whose venom has a marked local action, such as that of the genus *Lycosa*, form one group and those whose venom produces severe general symptoms but slight local reaction, like that of *Latrodectus mactans*, form the other (53). The reports that have appeared in recent years are concerned almost exclusively with the latter group. The genus *Latrodectus* contains three species that are largely responsible for the most severe accidents caused by spiders, namely, *Latrodectus mactans*, *Latrodectus indistinctus* and *Latrodectus hasselti*. In the western hemisphere *Latrodectus mactans* is the



most important poisonous spider from the point of view of the toxicity of its venom and also of distribution, which is very extensive. The distribution of *Latrodectus hasselti* (Australia) and *Latrodectus indistinctus* (Africa) is quite general in the eastern hemisphere. Smithers (54) found the latter species occurring in all sections of Cape Province, South Africa. Case reports involving these species were not encountered in the literature covered by this review.

The recent literature on *Latrodectus mactans* has been summarized by Sampayan (55) along with the presentation of new experimental data from his own work. Using macerated cephalothoraxes of *Latrodectus mactans* he found that 50 per cent of the guinea pigs (weighing 200 to 300 grams) given half of a cephalothorax subcutaneously or intravenously died, three-quarters of a cephalothorax killed all the guinea pigs injected. The glands from forty *Latrodectus geometricus* given intravenously were required to kill a similar sized guinea pig. Rats proved to be more resistant to the venom of *Latrodectus mactans* than guinea pigs. The venom from fifteen glands given intravenously was required to kill a rat weighing 185 grams. The white mouse was also more resistant than the guinea pig to the venom of the black widow spider. A rabbit bitten on the ear did not show symptoms but it should not be inferred that the rabbit is more resistant to the venom of this spider than the other laboratory animals used. The spider may have failed to inject any venom.

The toad is highly resistant to spider venom. The symptoms produced in the dog by spider venom are different from those observed in the guinea pig. Nausea, vomiting, diarrhea, excitability, trembling and howling are observed among dogs that have received sufficient doses. However, the dog is highly resistant to the action of the venom. Intravenous injections of 10 to 100 glands were necessary to produce death, which occurred in about a week. The results that I obtained (unpublished data) with guinea pigs and dogs are in complete accord with these findings. In my experiments one dog was given seventy bites by fifteen spiders. Pulmonary edema and pneumonia developed but the animal recovered after about ten days. The effect of spider venom on the lungs of guinea pigs closely resembles that of various snake venoms, histamine and fatal anaphylaxis, since in all cases severe emphysema is present (56). Among dogs extensive congestion of all the digestive organs, gastric hemorrhage and petechial hemorrhages in the terminal portion of the rectum were found. The only treatment that was satisfactory was specific antiserum. Epinephrine, ephedrine, atropine, ergotoxine, coramine, phenobarbital and morphine were administered subcutaneously. Morphine alone seemed to be of some value. Injections of calcium gluconate and magnesium sulfate were not effective (55). In my experience calcium gluconate gave symptomatic relief to pups that showed severe reactions to many bites of *Latrodectus mactans*.

Among dogs that had been bitten repeatedly by *Latrodectus mactans* a high degree of immunity developed. Although there was practically no reaction at the site of the bites initially, as immunization proceeded large wheals appeared around the site of each bite.

Shapiro, Sapeika and Finlayson (57), using the venom of *Latrodectus indistinctus*, performed experiments on frogs, guinea pigs, rabbits and cats. Solutions

of 1 6,000 caused marked slowing and finally complete arrest of the frog's heart. Injections of 1 to 3 mgm. into the ventral lymph sacs of frogs weighing 21 to 25 grams caused death in twenty-four to thirty hours. Electrocardiograms of the cat following injections of the venom showed almost immediate cardiac slowing, inversion of the T wave and absence of the P wave. The symptoms among cats and rabbits were increased blood pressure, restlessness, dyspnea, salivation, weakness, immobility and death. Necropsy did not reveal anything macroscopically abnormal. The smooth muscle of the small intestine and of the pregnant, but not the virgin, uterus was stimulated.

Odoriz and Sampayo (58) made an electro-encephalographic study of the effect of *Latrodectus mactans* venom on the central nervous system of the guinea pig, cat and dog. They found that the resulting tracings indicated hyperexcitability and resembled the effects of alkalosis and epileptic convulsions.

The majority of papers concerning poisonous spiders that have appeared during the last five years are reports of cases of black widow spider (*Latrodectus mactans*) bite. It is now well recognized that the bites of certain spiders may cause serious symptoms and very rarely death in man. The symptoms may simulate those observed in acute abdominal conditions and, unless arachnidism is carefully considered, an unnecessary operation may be performed on the patient. The importance of differential diagnosis is emphasized. The absence of abdominal tenderness and the presence of movement of the abdomen with respiration help one to distinguish arachnidism from an acute abdominal condition (59, 60, 61, 62, 63, 64, 65). To the classic symptoms of arachnidism caused by the bite of the black widow spider—increased pulse rate, increased blood pressure, abdominal rigidity and painful cramping of the muscles of the extremities—has been added burning of the soles of the feet, which is said to be almost invariably present. Use of mosquito bar netting by troops in the field is recommended as an effective measure against arachnidism (66).

Nonspecific treatment, such as the intravenous administration of calcium gluconate or magnesium sulfate, has not been universally satisfactory. As in cases of scorpion poisoning, prompt and lasting benefit has been obtained by the use of specific antiserum (64, 67), which is the treatment of choice. The sooner it is given after a bite, the better the results. It might be suggested that antiserum be given in cases of doubt as to whether arachnidism or an acute condition of the abdomen is the cause of symptoms. Should prompt relief of symptoms follow this procedure, the evidence would be strong that arachnidism was present. Such a difficulty might easily arise in the case of infants. Antiserum against the venom of the black widow spider has been produced recently in rabbits (68).

Kirby-Smith (64) reported one fatality among the twenty four cases that he reviewed. Unfortunately, necropsy was not permitted. A report by Beasley (69) of the pathologic effects of fatal black widow spider poisoning is the only one in the literature to my knowledge. In this case there were no gross pathologic changes, there were chronic adenitis and acute hemorrhagic nephritis. With the possible exception of the adenitis these findings are in accord with findings in laboratory animals.

A rare complication following the bite of a spider of undetermined identity has

been reported, the patient was a child three years old (70). Aside from swelling in the region of the bite there was marked hemoglobinuria, the color of the urine being inky-black. The concentration of hemoglobin was 59 per cent, erythrocytes numbered 2,940,000 in each cubic millimeter of blood. Following intravenous administration of solution of dextrose and transfusion, recovery occurred.

**REPTILES** *Lizards* Since the fear of lizards is common, it may be reassuring to emphasize that only two species in existence are known to be poisonous. They are the Gila monster (*Heloderma suspectum*) and the beaded lizard (*Heloderma horridum*). There is not an authentic record of death in man caused by the bite of these reptiles and not a single reference was found to their bite or poison in the period covered by this review.

*Snakes* Among a world population of about 2,400 different species of living snakes, less than 200 are dangerous to man (1). An important record of the poisonous snakes and the incidence of snake bite in Central America has been compiled by Clark (71).

The preservation and sterilization of the venom of snakes are always a problem, since the venom is grossly contaminated when collected (72). Sufficient heating for sterilization destroys certain elements of the venom, filtration holds back active constituents, but the addition of suitable amounts of phenol or sodium merthiolate keeps the venom solution, if properly collected, bacteria-free (73). The venom of the rattlesnake and moccasin was kept for six months without loss of toxicity by the use of alkyl-dimethyl-benzyl-ammonium chloride (zephiran chloride), 1:50,000 concentration (74). Venom kept dry or in solution is known to decrease in potency with time. When venom of *Lachesis alternatus* was kept in the dry state for twenty-four to twenty-seven years, 5 to 173 times as much of the poison was required to produce death as was necessary when the same venom was fresh (75).

The chemistry of animal poisons was reviewed by Kellaway up to 1939 (5). Important contributions have been made in this field. The crystallization (76, 77) of the toxic principle of *Crotalus terrificus* challenged the old belief that each action of snake venom was owing to separate toxic principles, such as neurotoxins, hemolysins, hemorrhagins, cytotoxins and so forth. Since the blood coagulating and proteolytic activities were always found in the same fraction regardless of treatment, it was concluded that the two activities are due to the same protein, which shows the properties of an albumin. The crystalline material contained all of the neurotoxic and hemolytic properties of the venom, a fact which has led to the belief that these two actions are due to one toxic principle. This conclusion has been questioned by Ghosh and De (78), who reported that they had been able to separate partially the hemolytic from the neurotoxic principle of *Crotalus terrificus* venom. These workers have reported the isolation of the neurotoxic principle of the venom of the cobra (*Naja naja*) (79). The active principles of certain samples of the venom of *Vipera russelli* and *Bungarus fasciatus* were concentrated so that for the same nitrogen content the activity of the neurotoxin of the former was about 7.8 and 9 times greater respectively than in the crude venom. The neurotoxins were not always free from the hemolytic principle (80).

Attempts at fractionation of the hemorrhagic and hemolytic components of water moccasin venom have been made. Both principles were found associated with protein fractions (81). The enzymes of the venom of the cobra (*Naja naja*), Russell's viper (*Vipera russelli*), *Boa fasciatus* and *Echis carinata* have been studied (82). Each contains a proteinase, which shows optimal activity toward gelatin. The venoms from *Naja naja* and *Boa fasciatus* contain cholinesterase but venoms of *Vipera russelli*, *Echis carinata* and *Crotalus terrificus* do not. In Ghosh's opinion the neurotoxin in cobra venom cannot be identical with cholinesterase, since its destruction does not affect neurotoxic activity. A purified hemolysin from cobra venom was prepared (82). The chemical composition of the yellow and the brown venom of *Bothrops jararaca* was studied (83) but an appreciable difference in the toxicity and solubility was not found.

The influence of geographic location on the toxicity of the venoms of *Crotalus terrificus*, *Bothrops atrox*, *Bothrops neuwiedi*, *Bothrops jararacussu* and *Bothrops jararaca* has been studied. The venom of *Crotalus terrificus* of Argentina, Paraguay and Brazil regardless of the color (yellow or white) is nearly devoid of proteolytic power but has a strong neurotropic action, whereas the venom of this same species in Venezuela is markedly proteolytic but weakly neurotoxic. The same is said to be true of the venom of *Crotalus terrificus durissus* of Central America to an even stronger degree. If effective antivenin against these snakes is to be produced, venom from different regions must be used in the immunizing process (84). Antivenin prepared with the more neurotoxic (white) venom of *Vipera aspis* neutralizes both white and yellow venoms (85). Phisalix (86) and Césari and Boquet (85) reported that the colorless venom of *Vipera aspis* is largely neurotoxic as compared with the yellow venom, which contains all of the usual toxic principles. The effects of heating and short-wave radiation on the toxicity of the venom were described (87). There are in the venoms of *Vipera aspis* and *Naja tripudians* free flavins and flavins fixed to the protein molecule, which account for their fluorescence (88).

Many substances have been found to attenuate, inactivate or destroy the venoms of snakes (87, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100). The saponin nature of snake venom is denied (101). Cobra venom reduces the vitamin C content of the adrenals and livers of guinea pigs (102). Tiger snake (*Notechis scutatus*) venom inactivates co-enzymes through the action of a nucleotidase, which accounts for the inhibitory effect of the venom on glycolysis, fermentation and on certain dehydrogenases (103). The perfused limbs of toads become more edematous after addition of cobra (*Naja tripudians*) venom to the perfusion fluid than after the addition of *Crotalus* or *Bothrops* venoms. The power to produce edema is proportional to the hemolytic power of the venoms (104).

Biochemical studies on rabbits (105) and on man (106) following repeated doses of cobra venom gave essentially negative results.

De (107) tested the effect of different substances on the activity of cobra (*Naja naja*) hemolysin on the erythrocytes of the guinea pig. Optimal pH for hemolytic activity was 7.6. Accelerators and inhibitors of hemolysis were studied. Slotka and Forster (108) determined by standard methods the sulfur content of the venoms of *Crotalus terrificus* and *Bothrops jararaca*.

*Pharmacology of snake venom* Feldberg and Kellaway (109) and Trethewie (110) have shown that histamine and coagulable proteins were liberated by perfused tissues when the venom of various snakes was added to the perfusing fluid. Trethewie (110) and Kellaway and Trethewie (111) found a strict parallelism between hemolytic activity and capacity to liberate histamine and proteins, which they interpreted as indicating that the liberation of histamine and protein in cell injury is mediated by the formation of lysocytin. The injection of the venom of rattlesnakes into intact rabbits (112) and into rabbits and guinea pigs (56) produces a decrease of the histamine in the blood plasma. If death was rapid, the reduction of histamine was less than if death was slow. A striking similarity of the action of trypsin and rattlesnake venom has been described (56). Kellaway and Trethewie (111) have reported what they assumed to be adenylic compounds in the perfusate from the perfused liver, kidney and heart of the rabbit and the heart of the cat after injections of cobra (*Naja naja*) venom. One is inclined to wonder whether the secondary effects of snake venom poisoning, such as the liberation of various substances like histamine, adenylic compounds and enzymes, have been allowed to eclipse the primary effects of the venom on the cells which made possible the liberation of these injurious substances (113). It may be asked if these substances are not rather a measure of the initial severe injury of the cells of the organism by the venom rather than the cause of the physiologic effects that follow its injection.

1 *Effects on circulation* The fall in blood pressure produced by sufficiently large doses of cobra (*Naja bungarus*) venom according to Gautrelet (113) is neither cardiac nor central but is peripheral in origin and is analogous to the action of histamine and proteotoxic substances. The action is essentially on the capillaries. Amuchastegui (114) reported that the venom of *Naja tripudians* causes arterial hypotension and venous hypertension, which he interpreted as being due to myocardial insufficiency. This action was considered primary. Macht (115) expressed the opinion that the decreased blood pressure caused by cobra neurotoxin is secondary to paralysis of the respiratory center or, in other words, that the venom is central in action. The work of Kellaway and Trethewie (111) indicates that the action of cobra venom is not simple, that it affects adversely the cells of all the organs studied. An attempt to account for hypotension or any of the other physiologic effects on the basis of a single mechanism is oversimplifying the problem. Gottdenker and Wachstein (116) and Macht (115) obtained hypertensive effects by injections of dilute solutions of cobra venom. Gottdenker and Wachstein observed vasoconstriction of the vessels of the perfused ear of the rabbit but dilatation of the coronary vessels. Gautrelet (113) did not observe a change in the vessels of the ear of the rabbit with *Naja bungarus* venom. Sarkar, Maitra and Ghosh (117) stated that the neurotoxin of cobra venom, unlike the crude venom, does not have any definite action on the blood pressure of rabbits.

Electrocardiographic effects of intravenously administered cobra (*Naja naja*) venom in rabbits were bradycardia, increased P-R interval, RST deviation and terminal heart block, in cats, tachycardia, inversion of T wave, extrasystoles,

increased P R interval (irregularly), effects resembling bundle branch block and ventricular fibrillation (111) Similar results were reported by Amuchastegui (118), who used *Naja tripudians* Gautrelet (113) observed only a few extra systoles resulting from injections of the venom of *Naja bungarus* into the dog Gottdenker and Wachstein (116) observed profound changes in the electrocardiogram of cats similar to those described by Kellaway and Trethewie (111) The differences in results that have appeared in the papers on cobra venom just referred to are not surprising when the species, dose of venom used, experimental animal, experimental procedure and other variable factors are considered. Rather, these differences tend to emphasize the complex nature of the problem In experiments with the venom of *Crotalus terrificus* on dogs, Soaje Echague (119) found such marked electrocardiographic changes in the rhythm and conduction of the heart that he concluded that the venom acted directly on the myocardium A comparison of the effect on goldfish of morphine, cobra venom and neurotoxin showed morphine to be a depressant or to be lethal, while cobra venom stimulated the activity of the goldfish (120)

Action of the venom of *Bothrops alternata* on the heart and circulation of the dog has been described There was initially hypotension, then definite electrocardiographic changes following single lethal or repeated small doses of the venom Amuchastegui considered the primary effect as peripheral and the cardiac effects as secondary (114)

Grasset and Schaafsma (121) have studied the toxicity of the venom and the blood of the "boom-slang" (*Dispholidus typus*) The venom contains coagulant and proteolytic fractions which are antigenically different. In contrast to what is usually seen, the serum and blood of the snake are nontoxic.

In addition to the typical findings in poisoning with the venom of the pit viper Brown (122), working with the venom of the water moccasin (*Agkistrodon piscivorus*), observed neurotoxic activities which were believed to produce respiratory and vasomotor depression or failure in dogs. Evaluation of central and peripheral effects was difficult under the conditions of his experiments The isolated heart of the frog responds to cobra venom (*Naja naja*) by irreversible systolic contracture with signs of impairment of conduction, shortening of the duration of systole and changes in the QRS complex and T wave. Auricular strips of the hearts of rabbits and guinea pigs are thrown into systolic standstill by cobra venom (116) The purified neurotoxin separated from the crude venom of *Naja naja* causes principally augmentation of the force of the beat of the perfused heart of the toad. With sufficient doses there is irregularity with ventricular block, which may be relieved by washing the heart Cardiac arrest is not produced even by high doses in the heart of the toad, rabbit or guinea pig (117)

The venom of the water moccasin (*Agkistrodon piscivorus*) acts directly on the ganglia of the isolated heart of the frog In sufficient concentration there is preliminary stimulation followed by failure. If the concentration of the venom is sufficiently dilute, the heart is stimulated and its efficiency exceeds that of the controls There were muscular irregularities with all but the weakest solutions (123)

2 *Respiration* There is agreement among workers that cobra venoms or their neurotoxins when given in sufficient doses cause respiratory paralysis

3 *Blood coagulation, hemolysis and so forth* The venom of *Bothrops atrox* coagulates blood or fibrinogen solution in vitro but like many venoms when given intravenously it produces marked prolongation of clotting time with a reduction in prothrombin and fibrinogen (124) Kauer, Bird and Rezinkoff produced an antiserum in rabbits which neutralized the clotting properties of the venom in vitro and modified the effect of the venom in vivo Von Klobusitzky (125) reported the isolation of the coagulating principle of *Bothrops jararaca* The influence of temperature on the toxic principles of the venom of this snake was investigated by Taborda (126) The fraction causing clotting is suddenly decreased when heated to 60° to 70°C The proteolytic principle decreases suddenly at 70°C In a previous publication, methods were presented for standardizing the coagulant action of the venoms of snakes of the genus *Bothrops* (127) Clotting time, using *Vipera russelli* venom, has been studied by Page, de Beer and Orr (128, 129) in its relation to prothrombin concentration in human plasma The effect of lecithinized venom on prothrombin clotting time (128, 129) and the stability of thromboplastic-like activity under various conditions of storage (130) also have been studied

Hanut (131) observed the effect on coagulation of blood in vitro and in vivo of the venoms of *Bothrops atrox*, *Bothrops nummifera*, *Bothrops alternata*, *Bothrops neuwiedi*, *Bothrops lanceolata*, *Vipera aspis*, *Cerastes aegyphiacus*, *Crotalus terrificus*, *Daboia elegans* and *Naja tripudians* The venom of *Daboia elegans* was the most coagulant while that of *Crotalus terrificus* had either a coagulant or an anticoagulant action The venom of *Naja tripudians* was inconsistent in its anticoagulant action from one batch to another The mechanism of the various actions was discussed

Henry (132) has reported that reduced glutathione given either in vitro or in vivo possesses an action which is antagonistic to the anticoagulant properties of cobra (species not stated) venom and protects the animal against hypocoagulants

The coagulant action of *daboia* (*Vipera russelli*) venom is dependent on sufficient concentration of calcium ions, is independent of platelets, is synergistic with the action of tissue extracts, of platelets and of cephalin and is distinct from that of thrombin, prothrombin or thromboplastic substances and from that of trypsin, papain or the snake venoms that resemble those enzymes in their coagulant action The coagulant action of *daboia* venom appears to be exerted through an interaction with tissue extracts or with cephalin contained in such extracts (133)

Methods of separating toxic and coagulating factors of tiger snake (*Notechis scutatus*) venom are given and the coagulating factors of this venom are described The coagulating factor is nontoxic when given subcutaneously and the toxic factor is devoid of coagulating power Tiger snake venom and its coagulating principle behave like thrombokinas plus calcium, readily converting prothrombin to thrombin (134)

The action of moccasin (*Agkistrodon piscivorus*), cobra (*Naja flava*) and fer-

de-lance (*Bothrops atrox*) venom on egg yolks, free lecithin, cephalin and derivatives of these phosphatides has been studied by Chargaff and Cohen (135), who reported that in contrast to the thromboplastic effect of cephalin, lysoccephalin does not have any influence on the blood clotting mechanism

In a test of coagulative potency of seventeen commercially available products, nine were found to be practically inactive. The only products that were significantly active were those that must be used orally or locally. *Bothrops jararaca* venom under the conditions of these experiments gave a coagulation time of twenty-one seconds with normal plasma and thirty one seconds with hemophilic plasma. The venom of *Vipera russelli* used in full strength coagulated normal and hemophilic plasma in thirty seconds (136)

Venoms that have phosphatide but little if any coagulating properties (*Naja tripudians*) produce after injection into the dog powerful hemolytic properties which destroy masses of erythrocytes. During this first phase, which is very brief, the cells are very vulnerable to hypotonic solutions or to physical disturbance. With the destruction of the phosphatides the negative phase is reached. In this phase hemolysis progressively decreases. It is thought that this mechanism is important in the protection of the organism against snake venoms (137, 138)

The venom of *Vipera russelli* alone or plus lecithin has been used as the clot-accelerating reagent in the Quick test. By its use three types of hemorrhagic tendency were recognized: hemophilia, purpura hemorrhagica and prothrombin deficiency (139)

A common property of snake venoms is their ability to cause hemolysis. De (140, 141) has presented a method of purifying the hemolysin of cobra (*Naja naja*) venom. The resulting product was eleven times as potent as the crude venom. The hemolytic activity of crude venom and purified hemolysin was depressed or lost after these substances had been exposed to iodine, ferricyanide and hydrogen peroxide. De made the significant discovery that the venom and purified hemolysin could be reactivated by reduced glutathione, hydrogen sulfide, cysteine and other agents.

The toxic nature of the blood of snakes is well established but the discovery is new and interesting that blood of poisonous and nonpoisonous snakes when mixed with the venoms of *Crotalus adamanteus*, *Akistrodon piscivorus* and *Bothrops atrox* inhibits their hemorrhagic action but does not prevent their lethal effects when injected into mice. Such an antihemorrhagic principle was not found in serums of warm blooded animals (142)

Bertrand and Vladesco (143, 144) studied the effect of snake venoms on the blood sugar level of guinea pigs and rabbits. Cobra venom caused a concentration of sugar in the blood more than double that of the control. Since Feldberg (11) showed that injection of snake venom into the celiac artery caused a long sustained liberation of epinephrine, the hyperglycemia may be owing to that mechanism.

Macht and Macht have published extensively on the comparative effect of cobra venom and morphine on animals, under a variety of experimental condi-



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are required to administer them. Githens and Wolff (161, 162, 163) have been concerned with this problem and the results of their work have answered many difficult questions. An immunizing mixture which contains a small number of rattlesnake venoms produces an antivenin with neutralizing power for the venoms of many other pit vipers, however, the North American pit vipers form three antigenic groups. Therefore, if an effective polyvalent antiserum is to be produced, venom from each group should be used in the immunizing mixture. It is particularly essential that one of the more neurotoxic venoms, such as that of *Crotalus mitchelli*, *Crotalus tigris* or *Sistrurus catenatus*, be included. Certain bothropic venoms and the venoms of true vipers are neutralized to some extent by pit viper antivenin.

Githens (164) has found the necrotizing factor much more constant than the neurotoxic factor of pit viper venoms but different lots of venom differ widely in their content of blood clotting factor, some lots showing more than 1,000 times as much as others, and the clotting factor does not bear any relation to the other actions of the venom. Polyvalent antivenins neutralize the necrotizing and neurotoxic factors more effectively than the clotting factor. These results are interesting in the light of the work of Slotta and Fraenkel-Conrat (76), who stated that the coagulating fraction of *Crotalus terrificus* venom contained all of the proteolytic activity while all the neurotoxic and hemolytic activity was contained in a single uniform fraction, which they crystallized. Important information on polyvalent antiserum has also been published by Grassett and Schaafsma (165, 166) and by Klobuatzky (167). Considering the variability of the venom of even the same species of pit viper one should be charitable if another investigator's results are different. The importance and wisdom of developing other adequate methods of standardizing antivenins appear obvious (168, 169, 170).

Snake venoms, when treated in a wide variety of ways or with many different substances, lost their toxicity but Lousseur (171, 172, 173) has shown that the treatment of snake venom with copper sulfate renders it nontoxic when injected into rabbits. However, the toxicity can be restored by proper treatment with proteins in vitro (141). An important question that remains unanswered is whether the antigenic power of the treated venom is retained. This information would be valuable also with regard to the venom detoxicated by glutathione (174, 175).

The temporary resistance, or refractoriness (tachyphylaxis), to the venoms of the cobra (116), *Crotalus adamanteus* (8), *Crotalus terrificus* and *Bothrops alternata* (7) has been described but a satisfactory explanation for this phenomenon has not been made.

An important observation (176) has been made in respect to cross immunization of mice with the endotoxin of *Salmonella typhimurium* and the venom of *Agkistrodon piscivorus*. Seventy per cent of the mice immunized to endotoxin survived intraperitoneal administration of 0.2 mgm. of moccasin venom, 73 per cent of the mice immunized to the moccasin venom survived that dose of the venom. Eighty three per cent of the mice immunized to *Salmonella* endotoxin survived 8 mgm administered intravenously while 53 per cent of the mice immu

nized to moccasin venom survived that dose of endotoxin. These doses of endotoxin and venom were respectively 4 and 2 lethal doses. The suggestion is made that cross protection may be due to the presence in gram-negative organisms and moccasin venom of a common factor characterized by hemorrhagic antigenicity and a lack of serologic specificity. This could have been tested, at least in part, by the use of other pit viper venoms.

**Pathology** The pathologic findings in the dog following intravenous injections of lethal doses of the venom of the rattlesnake (*Crotalus horridus*) have been reported (177). Fidler, Glasgow and Carmichael (178) have described the pathologic changes produced by subcutaneous injections of rattlesnake (*Crotalus atrox*) venom into monkeys. The gross and microscopic findings are described and the probable cause of death is discussed.

**Treatment of snake bite** The time-honored first step in dealing with snake bite is placing a tourniquet if the wound has been received on an extremity (179). There is not full agreement as to what the tourniquet accomplishes but that the purpose of applying the tourniquet is to retard the absorption of the injected venom few would doubt. How the tourniquet should be applied is a subject for debate. Kellaway (180) recommended obstruction of arterial flow but the usual instructions warn against such a procedure. Allen (181) presented a convincing experimental argument against an occlusive tourniquet after snake bite; he expressed the belief that it is wholly harmful and should be abandoned, the only exception is that it may be serviceable sometimes as a preliminary to a delayed amputation. The work of Duran-Reynals (182) and Madinaveita (183) has demonstrated that the venom of the members of the family Crotalidae (pit vipers) contains a spreading factor capable of increasing the permeability of connective tissue and blood capillaries. The venom of the Colubridae *Proteroglyph*a (cobra) family has relatively a small amount of this factor and the factor is almost entirely absent from the venom secreted by the skin of the toad. The spreading factor acts in the absence of the circulation and even aids in the diffusion of India ink in a dead animal. This work would seem to suggest that a tourniquet should be better able to control the spread of the venom of the cobra and related forms than the venom of snakes whose poisons are more necrotizing.

The next step usually suggested in treating a snake bite is incision of the fang marks and application of suction after the manner of Jackson (184). This method in the absence of antivenin therapy or in combination with it is of definite value in the opinion of too many observers to mention. Complete excision is applicable only if carried out within the first few minutes after the bite (180, 181). Amputation may be advisable if there is little doubt that a fatal dose of venom has been injected, as might be the case following the bite of a king cobra (181). Local venesection is recommended if done within two hours after the bite but not at all if a properly applied tourniquet has not been in place (180).

In the symptomatic treatment of snake venom poisoning many drugs may be applied locally or given systemically with doubtful effect on the outcome (48, 185).



diarrhea and pain of injection, were mentioned. The Council stated that cobra venom must not be recommended for those who are severely ill except those suffering from inoperable malignant tumors or from incurable disease.

Moccasin snake venom (species not stated) was used in treatment of herpes simplex (222).

The venom of *Bothrops atrox* was used by Hanut (223) as a hemostatic agent.

The venom of *Vipera russelli* was employed as a hemostatic agent by Page and Thomas (224).

Alvaro (225) has reviewed the use of snake venom in ophthalmology, in which it has, judging by his report, rather a limited application.

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## NEUROSECRETION

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Specific functions require specific structural elements. The transmission of nervous impulses, for instance, is accomplished by specialized types of cells which, as a rule, are unmistakably characterized by their shape and structure. If anywhere in an organism cells with dendrites and axons, Nissl bodies and neurofibrils are found, no experimental proof is required to show that such elements are neurons and are concerned with the conduction of nervous impulses.

Similarly the process of "secretion" is reflected in the structural peculiarities of the gland cells. It has been shown by the work of Langley, Ranvier, Altmann, Heidenhain and many others (11, see Bowen, 1) that the products of most gland cells first become visible as granules which grow, undergo various kinds of transformations, and are eventually discharged. This process is essentially similar in gland cells whose products serve functions as different as, for instance, the pancreas of the mouse (20), the ink gland of the squid (36), or the cutaneous poison glands of the newt (19). It follows that for the investigation of secretion as an intracellular process it is not necessary to establish first the functional significance of the secreted material. Gland cells may be described, therefore, along purely morphological lines, but a description of this kind is significant only to the extent to which it makes possible the identification of such cells as sources of physiologically active substances.

It appeared justified, therefore, to formulate the concept of "neurosecretion" before any physiological effects of the material produced by the cells were known. It evolved from the observation of nerve cells which resemble gland cells in that they show the cytological features of glandular activity, i.e., they produce and discharge granules and colloidlike material. These products of the nerve cells show the characteristics of protein substances (62, 12, 65). On the basis of these observations hormonelike effects of nervous tissue became a matter of considerable interest. It is certainly strange that cells as highly specialized as nerve cells should also have the faculty of acting as gland cells, and many aspects of neurosecretion, particularly as it concerns mammals and man, are still obscure. However, a number of facts are well established and will be reviewed here. They concern the occurrence of neurosecretory cells in invertebrates and vertebrates, the cytology of the secretory cycle, and data on the functional significance of neurosecretion.

**OCCURRENCE OF NEUROSECRETORY CELLS** Glandlike nerve cells occur in invertebrates and vertebrates. In invertebrates neurosecretory cells have been observed in worms, molluscs and arthropods.

The polychete worm *Nereis* possesses two symmetrically arranged groups of secreting nerve cells (fig. 1A) in the dorso-caudal portion of the cerebral ganglion (40). In *Aphrodite* almost one half of the cerebral ganglion is glandular. The dorsal region of the cerebral ganglion of the earthworm (*Lumbricus terre-*

tris) contains a great number of such cells. They are also found in the leech (*Hirudo medicinalis*) (41, 59)

Many species of molluscs have been studied, but nerve cells containing secretory granules were found only in the opisthobranchiates, where they are very conspicuous in *Aplysia* (Tethys) and *Pleurobranchaea*. In the dorsal portion of the cerebral ganglion of *Aplysia* two well outlined groups of secreting cells are arranged symmetrically on both sides of the midline (fig 1 B). Neurosecretory elements occur also in the posterior visceral ganglion of *Aplysia*. In *Pleurobranchaea* the glandlike area lies in the caudal portion of the cerebro-visceral ganglion (39). Similar, but usually less conspicuous neurosecretory cells are observed in *Doris*, *Doridium*, *Aeolis*, *Philine* and others (41, 59)

Signs of neuroglandular activity are also found in various groups of arthropods. Neurosecretory cells containing large colloid inclusions constitute a considerable

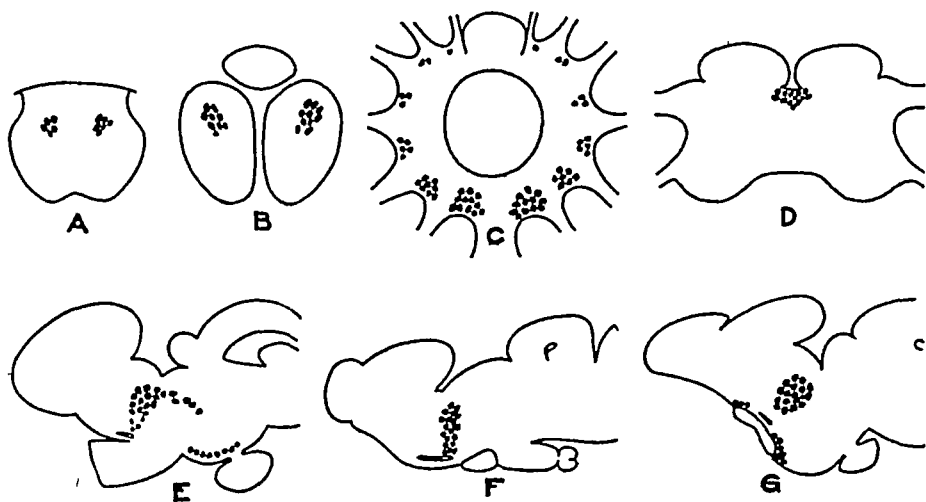


Fig 1 Diagrams illustrating the localization of secreting nerve cells in sections through the central nervous systems of various invertebrates and vertebrates. A, worm (*Nereis*), B, snail (*Aplysia*), C, horseshoe crab (*Limulus*), D, insect (*Leucophaea*), E, fish (*Tautoga*), F, amphibian (*Bufo*), G, reptile (*Lapemis*)

proportion of the central nervous system of *Limulus* (44) (fig 1 C). In insects the pars intercerebralis of the cerebral ganglion contains secreting nerve cells (fig 1 D). They have been described in Hymenoptera (65, 41), Hemiptera (16, 66), Orthoptera (42), Lepidoptera (10), Coleoptera, Neuroptera, Trichoptera and Diptera (11, 64). Aside from the pars intercerebralis neurosecretory cells are found also in the frontal ganglion, the subesophageal ganglion, and in certain abdominal ganglia (42, 10).

It is interesting that Hanström (17) derived the glandular component of the pars intercerebralis from the paired frontal organs of lower insects. In the ascending series of insects these sensory centers become secretory in nature and are incorporated into the central nervous system. A similar change of function occurred in crustaceans where the glandular X-organ of the eyestalk developed from the lateral frontal organ, a structure which, according to Hanström (18)

is homologous with the paired frontal organs of insects. In view of these findings the gradual incorporation of the neuroglandular "cerebral organ" of nemertean worms into the central nervous system assumes renewed interest (43).

Of the vertebrates fishes and amphibians have been investigated extensively, while reptiles and mammals have not received equal attention. No data are available regarding birds. A considerable number of human brains, however, have been carefully studied.

In the fishes where nearly 100 different species have been examined, five different areas within the central nervous system are either definitely known to secrete or their cells at least resemble secreting nerve cells. These groups are the following:

a The nucleus of the nervus terminalis in *Xiphias gladius* and *Tetrodon lagocephalus* (54) consists of a group of autonomic nerve cells which have the structural peculiarities of secreting elements, but whose glandular activity has not been studied in detail. We shall not refer further to this cell group.

b The nucleus preopticus, pars magnocellularis, of the diencephalon (fig. 1 E) contains the most active cells which have been studied in many species (46, 47, 48, 53, 54, 55). The nucleus preopticus of the fishes is homologous with the nuclei supraopticus and paraventricularis of the reptiles and mammals. The description of neurosecretory cells in this review will to a large extent deal with the cells of the preoptic nucleus.

c The nucleus lateralis tuberni in the hypothalamus (fig. 1 E) does not occur in all species, but is of particular interest in some (48). It has been shown that the cells of this nucleus operate in cycles paralleling the seasons (54). This cell group will also be discussed with reference to the participation of the nucleus in the elaboration of the secretory material (52, 31).

d In a few families a small area occurs in the tegmentum of the midbrain whose cells have been found to form colloid (49), but little else is known about this group and it will not figure prominently, therefore, in the following.

e Around the central canal in the caudal half of the spinal cord in elasmobranchs and in some teleosts very large and highly interesting cells, first mentioned by Dahlgren (8), were interpreted by Speidel (62, 63) as glandlike nerve cells. Speidel was the first to formulate this concept on the basis of a large amount of material. It is difficult to understand why Speidel's work did not receive more attention.

In the amphibians the nucleus preopticus (fig. 1 F) of the toad (*Bufo*) contains active neurosecretory cells which differ little in the various species (50, 38). By contrast the frogs show much less activity, and all urodeles are very poor in this respect.

The reptiles possess two secreting nuclei, the nucleus supraopticus and the nucleus paraventricularis (fig. 1 G) which are both derived from the nucleus preopticus of the fishes and the amphibians (28). In the lizards as in the urodeles granules are rarely found, but in turtles and snakes the cells are very active (51, 25).

The same two nuclei as in the reptiles are found in the mammals. Secretory activity which is often not very impressive has been observed in a number of

species such as the opossum (21, 60), various rodents, dog and monkey (67, 14, 29, 30)

All the findings so far mentioned concern cell groups in the central nervous system. In the case of the preoptic nucleus and its homologues in the hypothalamus the cells belong to the autonomic system. A search was made, therefore, for signs of secretory activity in peripheral ganglia of the autonomic system, indications of which were found, however, only rarely (26). In the cells of the myenteric plexus of rats Ito and Nagahiro (22) described granules which they consider as secretory.

There is considerable interest in the question whether secreting nerve cells occur in the human nervous system. The cells of the nuclei supraopticus and paraventricularis of the human hypothalamus have in the past impressed the observers as "pathological". The nuclei of these cells are commonly eccentric, the Nissl substance lies in a marginal zone of the cell body in a manner similar to that of chromatolytic cells, and the cytoplasm is often vacuolated (15). To this description must be added that the capillary bed of the two areas is extremely dense, and that the blood vessels are often intimately associated with the nerve cells, forming pericellular baskets, or even entering the cell bodies as endocellular vessels. These peculiarities are subject to considerable variability which concerns particularly the number of the nuclei in the cells. While in one case no cell appears to contain more than one nucleus, binucleated cells may abound in other cases. Occasionally cells with more than two nuclei are observed. Furthermore the nuclei are not always vesicular, but may be lobated or invaginated. Likewise the degree of vacuolization of the cytoplasm varies. Some of the vacuoles contain a colloidlike material, but the majority of them appear empty, they may contain lipoids (34, 35). Acidophil granules are infrequently found in greater or smaller numbers in the marginal Nissl zone. Also the axons may contain a few droplets of colloid (51, 14).

The similarity of the structural peculiarities of these cells in man to the characteristics of secreting nerve cells in animals is obvious. Still more important is the fact that the cells of the supraoptic and paraventricular nuclei are homologous with those of the preoptic nucleus of lower vertebrates (6). The nucleus preopticus becomes separated into two cell groups in the reptiles, but even in man a connection still exists. It seems reasonable to interpret the cellular peculiarities of these two nuclei in man as due to a secretory activity rather than to pathological conditions.

We are not venturing any speculations concerning the possible rôle of the glandular activity of hypothalamic centers in the human brain. In about 200 cases, studied by several investigators (56, 12, 37, 13, 14, 33), no definite relation was found between the histological picture of the supraoptic and paraventricular nuclei on the one hand and conditions of the individual at the time of death on the other hand. No correlation could be established between age, sex, terminal disease, time of the year, etc., and the histological appearance of the cells of the nuclei supraopticus and paraventricularis.

*Conclusion.* From all these data the fact emerges that cells which combine the structural characteristics of nervous elements with those of gland cells are

a common occurrence in invertebrates and vertebrates. They are found in well known nuclei of the central nervous system as for instance the pars intercerebralis of the insect brain, or the nucleus preopticus of the fishes and amphibians and its homologues, the nuclei supraopticus and paraventricularis, of the reptiles and mammals.

**CYTOLOGY OF NEUROSECRETORY CELLS** The cytology of glandular secretion in general is concerned with the cycle through which a gland cell passes from the first appearance of secretory granules to the extrusion of the product. In order to understand the significance of a secretory process it is important to know as much as possible about the origin of the material secreted by the cells and the cellular constituents which participate in its elaboration.

In the beginning of a secretory cycle the cytoplasm of a gland cell is free of granules. "These presently appear in ways characteristic of different glands, and increasing in number and size give to each gland cell its characteristic histological appearance" (Bowen, (1), p. 308). After the contents of the cell have been expelled, the cell may sooner or later enter upon a new cycle. This process of secretion is essentially similar in most gland cells and has been studied extensively with histological methods, supplemented by observations of living material. Its general validity is well established.

Practically all cellular constituents have at one time or another been considered as instrumental in the synthesis of secretory granules (9). At present most investigators agree that in many gland cells the Golgi apparatus plays an important rôle in the elaboration of the secretory material as manifested by the close spatial relationship between the Golgi apparatus and the granules, and by the changes the Golgi material undergoes during their formation. But there are also cases of glandular activity where a participation of the nucleus cannot be denied. The rôle of the mitochondria and of the "ergastoplasm" is undetermined (1).

The cytology of the neurosecretory cycle has been studied in fishes and amphibians. The granules do not appear to be formed in association with the Golgi apparatus or with the mitochondria. Three modes of elaboration of neurosecretory granules have been observed (58). Apparently they originate a, within the Nissl substance, b, within a basophil portion of the cytoplasm, or c, within the nuclei (fig. 2).

The Nissl substance of the cells of the preoptic nucleus is characteristically located in the peripheral zone of the cell body. The smallest secretory granules are observed in the same region of the cell in close association with the Nissl bodies which they eventually replace (fig. 2 A). The observation that the Nissl bodies diminish while the granules increase may mean that the Nissl bodies contribute in some way to the growth of the granules. However, what actually happens during this process cannot be determined by cytological methods. By far the largest percentage of secreting nerve cells appear to operate according to this mode. In the nucleus preopticus of most teleosts and of all amphibians which show secretory activity, the granules appear to originate in association with the Nissl bodies. This seems to hold true also for the secreting cell groups in reptiles and mammals for which fewer observations are available.

A strongly basophil cytoplasm is in some cases found close to the nucleus, and fills, for instance in catfishes, deep invaginations of the nuclear membrane. Also within this basophil region of the cytoplasm which is not identical with the Nissl substance, acidophil granules may be found (fig 2 B). This manner of elaboration plays probably only a small rôle. In fact, it has been observed so far only in the preoptic nucleus of a few species of teleosts.

In the third type acidophil granules are observed within the nuclei (fig 2 C). One cannot escape the impression that there is less chromatin in nuclei with many than in those with few or no acidophil granules (31). However, as in the case of

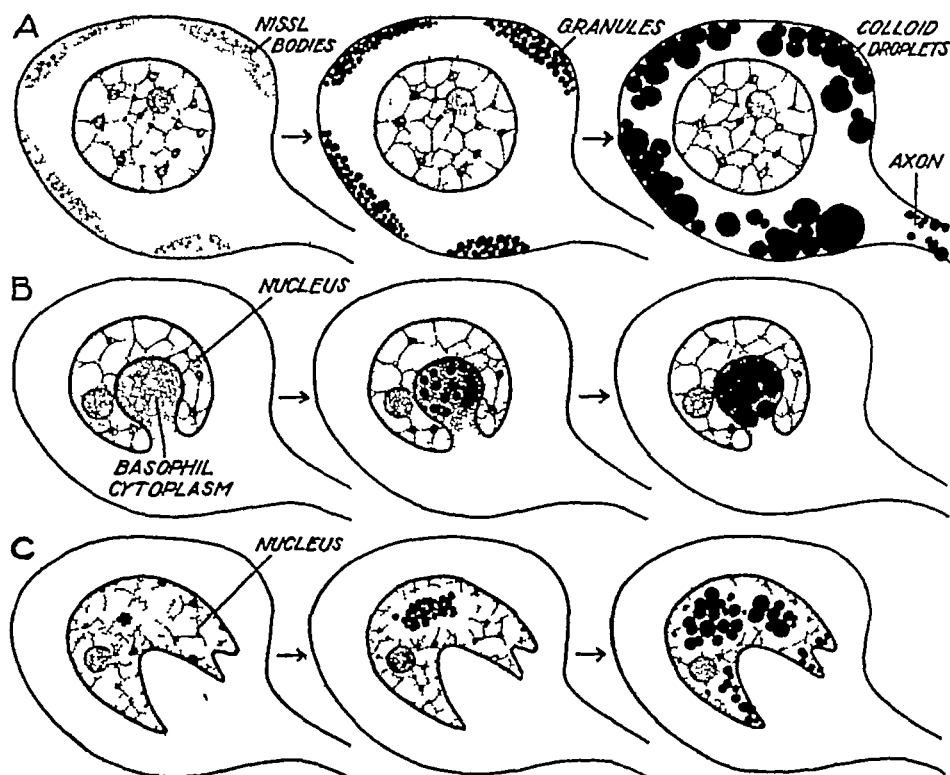


Fig 2 Diagrams illustrating three types of origin of neurosecretory granules. A, the granules originate in association with the peripherally located Nissl bodies. B, the granules originate within an invagination of the nucleus filled with basophil cytoplasm. C, the granules originate within the nucleus.

the Nissl bodies the study of cytological preparations alone does not permit a statement with regard to the antecedents of the intranuclear granules. Of this type only a few instances are known. It occurs in the nucleus lateralis tuberculi of certain fishes, and also in the preoptic nucleus of catfishes.

Evidently neurosecretory granules at the time of their first appearance are associated with basophil constituents of the cells, in most cases with the Nissl bodies, in fewer instances with basophil cytoplasm and chromatin. The significance of this fact depends upon the chemical relationship among the basophil substances (5, 7). If they have something in common that plays a rôle in the elaboration of the acidophil granules, essentially the same chemical processes

could take place whenever granules develop in association with any one of the basophil substances

Such a concept would help to explain certain seemingly inconsistent cytological observations. For instance in *Tautoga onitis*, a teleost fish, the secretion in the nucleus lateralis tuberculi takes place in the region of the Nissl bodies. In this species it can be clearly shown that the Nissl bodies disappear as a stainable material while the secretory product is formed. By contrast, the cells of the same nucleus lateralis tuberculi in catfishes, *Ameiurus nebulosus* and *Noturus flavus* (31), mainly show nuclear secretion in which the chromatin diminishes with increasing accumulation of granules. The few instances where all three types of secretion occur simultaneously within one cell group, such as the preoptic nucleus of the catfish, can be viewed on the same basis as not essentially different from the many cases where only one type is found.

Preliminary investigations indicate that we can expect to find in opisthobranchiate snails similar modes of elaboration of neurosecretory material. Also higher vertebrates which have been studied less thoroughly than fishes and amphibians, appear to follow a scheme corresponding to that of the lower vertebrates.

There is evidence that the majority of the cells of the nucleus preopticus of *Fundulus* or of *Bufo* pass through about the same stage of secretory activity at the same time. One will find, therefore, in one specimen predominantly small granules in the marginal Nissl zone. In another specimen the granules of most cells may be larger. Evidently they grow and coalesce to form larger droplets. In this stage in *Fundulus* or *Bufo* the granules lose their relation to the Nissl bodies and gradually occupy more space than that available within the Nissl zone. Finally they fill the cell so completely that the nucleus may come to lie eccentrically as is often the case in other gland cells. If enough cells contain large droplets staining brilliantly with acid fuchsin, the area of the preoptic nucleus may appear red when a section of the brain is viewed with a low power magnification, whereas in the rest of the brain the cells take the nuclear stain only. Such an area of secretory nerve cells looks much more like glandular than nervous tissue.

The increase in size is the only visible change the granules undergo during the process of secretion, the stainability is the same in large and in small granules. Fixation and staining are not required for the demonstration of the granules and droplets, they can easily be seen in fresh smears of brain tissue containing the preoptic nucleus of *Fundulus* or *Bufo*.

In some cases, for instance in the teleosts, *Fundulus* (55) and *Cristiceps* (53), the granules lie frequently within vacuoles which may grow to considerable size. Vacuolization of the cytoplasm is also observed in reptiles and mammals, but not in amphibians and also not in most fishes. Its significance is undetermined.

In certain animals the cells of the hypothalamic nuclei do not produce granules. Instead, the basophil Nissl substance diminishes in proportion to the increase of a homogeneous acidophil material. This process which is typical of the cells of the supraoptic and paraventricular nuclei of certain snakes and of the dog



should be considered as equivalent to the formation of granules and droplets. A closer study of this type of secretion is under way.

The fate of the material secreted by the nerve cells is not fully known. In a primitive type of neuroglandular organs, that of the nemerteans (43), a duct exists through which the products of the secreting elements can be discharged. In all other cases no duct exists, and the secreted colloid which is often observed lying between the cells which produce it, may be carried off by the blood stream. In this respect secreting nerve cells can be considered akin to the cells of the adrenal medulla with the difference that they are fully differentiated nerve cells which in addition have acquired a glandular character.

There is another, very peculiar pathway through which the products of secreting nerve cells may be discharged. The same acidophil droplets which are formed within the cell body are also often seen along the axon, for instance in molluscs (39). In insects a nerve that arises from cells of the *pars intercerebralis* and which innervates the corpus cardiacum and probably also the corpus allatum may contain considerable amounts of colloid. Also in fishes, such as the tench (54) and the catfish, acidophil droplets can be traced all along the axons from the cells of the preoptic nucleus to the hypophysis. These droplets may in some cases be so numerous that the preoptico-hypophyseal tract can be differentiated from other fiber connections on account of the acidophil granules in much the same way as a fiber tract can be traced in a Marchi preparation (32). It is of interest that in both cases, the insects and the fishes, the colloid formed by groups of neurosecretory cells is directed toward an endocrine organ which itself is composed of nervous and glandular components (45).

*Conclusion.* The view that the nerve cells under consideration are truly secreting elements may by now be considered as sufficiently well documented. It can be checked with simple histological methods in easily obtainable animals such as the toad (*Bufo*) or the killifish (*Fundulus*). Indeed no other interpretation has as yet been offered by any observer of these cells.

**FUNCTIONAL RÔLE OF NEUROSECRETORY CELLS.** The problem of the physiological significance of neurosecretion has been attacked in two ways: *a*, the secretory activity has been studied in relation to cycles in the lives of the animals, *b*, endocrinological methods have been applied to the nervous tissue which contains secreting nerve cells since it was assumed that the histological findings indicate a sort of internal secretion.

As an example of a correlation between neurosecretory activity and phases in the life cycle, the honey bee may be cited. The worker bee exhibits the greatest amount of colloid droplets in the *pars intercerebralis* during the middle period of its life when it starts to collect nectar and pollen. Freshly emerged and very old workers show little if any secretion. By comparison less colloid is found in the *pars intercerebralis* of the queen and still less in that of the drones (65). Generally female insects show more neurosecretory activity than males. It becomes less conspicuous with age also in insects other than the honey bee, for instance in the moth *Cecropia* (11). In *Limulus* the largest females seem to possess a greater number of neurosecretory cells in proportion to the total number of nerve cells than smaller females and males (44).

The seasonal cycle of the nucleus lateralis tuberculi of certain teleosts serves to illustrate the relationship between neurosecretory activity and the functional state in the vertebrates. In the tench (*Tinca vulgaris*), a close relative of the carp, the activity of the cells of the nucleus lateralis tuberculi ceases during the winter months, while it is at its height during the summer months (54). This finding, however, does not apply to the nucleus lateralis tuberculi of the catfishes *Noturus flavus* and *Ameiurus nebulosus*, in which no correlation between time of year and neurosecretory activity could be demonstrated by observations extending over several years (57).

What histologically looks like variations in the secretory activity of the cells thus coincides with certain phases in the life of the organism. It may be suggested, therefore, that the glandlike activity of the neurons plays some rôle in the life cycles of animals such as the ones mentioned.

Relationships of this kind have not, however, been established in all cases. The secreting nerve cells of the snail *Aplysia* (*Tethys*), for instance, did not show any significant change from month to month in the course of a full year, neither did those of *Limulus* (44). Similarly the cells of the nucleus preopticus of *Fundulus* or *Bufo* may be engaged in secretory activity or be "at rest" at any time of the year. Each individual appears to follow its own cycle with respect to the secretion in the preoptic nucleus. All attempts to influence experimentally (for instance by injections of pilocarpin) the secretory activity of the glandlike nerve cells in *Aplysia*, *Limulus*, *Fundulus* and *Bufo* have so far failed.

Endocrinological methods have been applied to nervous centers in many instances. Positive results were obtained, however, only in invertebrates where two types of hormonally controlled processes can be correlated with the secretory activity of the central nervous system. The one concerns insect development, the other physiological color change in crustaceans.

It has been known for some time that the insect brain produces a hormone causing pupation in Lepidoptera (23, 24) and in Hymenoptera (61). But only Wigglesworth (66) succeeded in localizing the source of a corresponding hormone of Rhodnius (Hemiptera) in that part of the protocerebrum which contains the pars intercerebralis with its neurosecretory cells. Implants of this portion of brain tissue caused molting in the host, whereas other parts of the central nervous system as well as other tissues gave no results. Neurosecretory cells could still be identified as such when the implanted pars intercerebralis was checked histologically at the conclusion of the experiment.

A distinct chromatophoretropic action has been obtained with extracts from the central nervous system of worms (*Lumbricus*, 27), molluscs (*Venus*, 27), and arthropods (*Limulus*, various crustaceans and insects). In crustaceans extensive experiments with various separately tested areas of the central nervous system or with different extract fractions of one area gave qualitatively different results. Consequently the production of at least two active principles by the central nervous system has been postulated (2).

The chromatophoretropic effects constitute convenient tests but do not necessarily mean that in the donor these substances are concerned with color change.

They indicate, however, the presence of physiologically active substances whose specific rôle in the organism is not always known

There is considerable evidence that such chromatophorotropic substances are produced by secreting nerve cells. Chromatophorotropic extracts were, for example, obtained from the central nervous system of *Periplaneta* (4) which contains neurosecretory cells. Still more convincing are observations concerning the chromatophorotropic action of the central nervous ganglia of *Limulus* which differs quantitatively in various separately tested portions (3). These distinct differences correlate so well with the quantitative distribution of neurosecretory cells that these in all probability can be considered as the source of the chromatophorotropic principle (44).

*Conclusion* If it can be demonstrated in more instances, particularly in vertebrates, that neurosecretory cells produce active substances, the original definition of neurosecretion will become inadequate. Neurosecretion will then signify not only the histologically visible elaboration and discharge of granules and masses of colloid, but also the production of physiologically active substances by the same nerve cells which elaborate granules. In the study of the function of secreting nerve cells considerable interest lies in the morphological evidence of a secretion directed along the axons. In the insects the neuro-endocrine mechanism consisting of *pars intercerebralis* and *corpora cardiaca* and *allata* is concerned with the hormonal control of development, the hypothalamo-hypophyseal system could similarly account for sustained, as for instance seasonal, changes of pituitary activity in vertebrates.

#### SUMMARY

*a* Groups of nerve cells which pass through secretory cycles in the manner of gland cells occur in the central nervous system of invertebrates and vertebrates.

*b* In fishes and amphibians the secretory granules of nerve cells appear to originate in association with basophil constituents of the cells, i.e., chromatin, basophil cytoplasm, or Nissl bodies.

*c* In insects and fishes a peculiar pathway of the products of neurosecretory cells can be observed along the axons to glands of internal secretion.

*d* In two instances in invertebrates the origin of physiologically active substances has been traced to parts of the central nervous system which contain neurosecretory cells.

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# INORGANIC INDUSTRIAL HAZARDS

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Since a broad discussion of inorganic health hazards would be difficult to conform to within the confines of any short review, the following discussion may be regarded as supplemental information with reference to certain phases together with some necessary description of the mode of contact of workers with these materials. Industrial hygienists are well aware that coincident with advance in technology and industry and especially since the beginning of the war there has been increasing application of new or untried materials. Our knowledge of the toxicity of many of these substances is scanty at best and in certain cases industrial injury has resulted from ignorance rather than negligence. In addition to the use of new or untried substances, one finds new ways in which substances known to be toxic are used, or unforeseen ways in which toxic substances are introduced in manufacture which in turn often lead to damage or fatalities in industry.

The type of exposure which is most hazardous, with the possible exception of active skin poisons, is that of inhalation of dusts, fumes, or gases. Accidental ingestion of inorganic poisons is comparatively rare and modern industrial sanitation is therefore largely concerned with the control of air contaminants.

Many of the industrial poisons produce both acute and chronic effects. In the acute form, profound functional disturbances are caused by an overdose of the various blood and nerve poisons, in the chronic form, because of diminished dosage, toxic substances may cause disease of a progressive character by producing gradual histological changes of the blood and tissues of the body. In addition there are doubtless numerous instances in which minute quantities of poison are not sufficient to produce specific effects and yet do bring about a general deterioration and diminished power of resistance, as instanced by the prevalence of incidental disease amongst these workers.

A large amount of information has been accumulated with reference either to the medical or the medico-legal phase of poisoning by inorganic poisons, or to the therapeutic action of these substances, which is often of little use to the industrial hygienist. Therapeutic dosage as well as information relating to the effect of intravenous injection of many substances likewise is seldom of immediate value. Acute toxic effects of heavy exposure are usually the result of accident and may or may not give information of value concerning the effects of lower concentrations. The effects of long continued exposure to relatively low concentrations are the main concern of the modern hygienist. In the case of many substances, continued exposure of this type may prove to be a matter of no concern, for the amount absorbed may be well within the amount of individual physiological tolerance. Some industrial poisons are sufficiently cumulative however so that absorption of even small quantities may be a matter of some importance.

It is now recognized that there is a time-lag with many substances such as phosphorus or radium, which, absorbed in small amounts over a period of years may show no outward effects or symptoms until extensive, often irremediable, damage has been done. While substances of this type are fortunately rare or unusual, it is necessary to consider this point in evaluating certain of the newer materials of industry.

Historically and because of the large number of workers involved, lead poisoning occupies a prominent place in industrial hygiene. The lead industry itself is huge and its ramifications throughout industry in general are manifold.

Not only has the incidence of lead poisoning diminished in industry, the severe type of poisoning which has been described in detail in the past as occurring in industrial workers is very seldom evident at the present time, since the appearance of even mild symptoms in relation to a history of exposure usually receives prompt medical attention. When they do occur cases of lead poisoning are more likely to be found in small establishments arising from poor working conditions or ignorance of proper protective measures. The steady diminution in cases of fatal poisoning and also of milder forms of poisoning has been noted by Hoffman (1927) and has resulted from a more widespread appreciation of the potential hazard of exposure to lead or its compounds. In Great Britain according to Hunter (1943) less than 100 cases of lead poisoning were notified in 1938 as compared with more than 1250 in 1899. In addition, the substitution of machine methods for hand labor has in several cases resulted in putting an end to this disease in certain industries. Lead poisoning which was once common among file cutters has been eliminated where machine methods of file cutting have been introduced (Hunter, 1943).

While the incidence of lead poisoning has been lessened typical cases of industrial lead poisoning are still being reported from time to time among workers using red lead in stencilling glass before cutting and grinding (Lederer, 1935), from stencilling silk (Gerbis, 1935), among telephone and telegraph workers (Mytnik and Shevelukhin, 1936), in type setting (Lederer, 1936), from cutting tobacco on lead plates in cigar manufacture (Jordans, Zylmans and Broos, 1936), from lead dust in automobile body plants (Humperdinck, 1938), in the manufacture of lead bronze bearings (Weber, 1937), among solderers metal finishers, and welders in automobile plants (Molitor, Arnoldson and Hausser, 1938), from setting stained glass in lead (Rastelli, 1940), from the volatilization of lead from the painted metal in cutting structural steel with an oxyacetylene flame (Bata, Firket and Leclerc, 1940), and among soldiers exposed to fumes from field kitchens in which leaded gasoline was burned (Abraham and Baird, 1942)—to cite but a few instances.

The diagnosis of lead poisoning still appears to be a matter of some difficulty. No single criterion can be accepted as infallible. With chronic lead poisoning and a clear cut history of exposure diagnosis is often simple, with incipient lead poisoning it is frequently very difficult to make a true diagnosis of lead poisoning. A number of factors must be taken into account and each of these carefully evaluated for not only diagnostic skill and experience with lead poisoning are

necessary but careful and competent laboratory investigation of the blood picture and of the urinary excretion of lead is required

The cardinal symptoms of lead poisoning include colic, the lead or Burtonian line, basophilic stippling of the erythrocytes, pronounced urinary excretion of lead, palsy, and anemia. Any one or several of these may be absent. The absence of only one of these symptoms according to Pfeil (1941) makes the diagnosis doubtful. A great deal of weight has been, and still is, attached to the urinary excretion of lead in lead poisoning. High values indicate active absorption, but a single low value does not necessarily indicate the absence of lead poisoning. In doubtful cases it is advisable to determine the daily urinary excretion on two or more consecutive days.

The absorption of lead is greater following inhalation of dust or fume than that which occurs following the ingestion of lead compounds. Minot (1924) found that the quantity of lead present in the tissues of animals twenty-four to forty-eight hours after administration by lung of 150 to 200 mgm of solid lead carbonate is as great as after the administration of several grams of lead in solution during weeks of continuous lead feeding. Fairhall, Sayers and Miller (1940) showed that absorption of lead from the lungs is related to the solubility of the lead compound. For instance, less lead is absorbed and distributed throughout the organism following the inhalation of lead phosphate than occurs in the case of lead monoxide. The latter is relatively very soluble in the tissue fluid of the lungs, whereas lead phosphate is very insoluble in such a medium. The low incidence of lead poisoning amongst miners in galena mines is probably due to the similar insolubility of lead sulphide. In fact, owing to the almost complete absence of lead poisoning among galena miners in Bavaria, Rambousek (1913) recommended a soluble sulphide soap for lead workers as a prophylactic measure.

The close parallelism between the metabolism of calcium and lead was first definitely demonstrated by Hunter and Aub (1926), who found that repeated injections of parathyroid hormone in patients with chronic lead poisoning resulted in increased excretion of both lead and calcium following the rise in serum calcium. The demonstration of this relationship of lead and calcium has proved to be of fundamental importance with reference to our knowledge of lead poisoning.

Calculation by Kehoe, Cholak, Hubbard, Bambach and McNary (1943) following the administration of soluble lead to two individuals indicates that if the oral intake of soluble lead does not exceed half to six-tenths of a milligram per day, lead accumulation in the body will not occur, but a balanced metabolism takes place.

The rate of absorption of lead from the digestive tract is stated to be especially rapid during the first twenty minutes (Pogato and Antonoli, 1940) then diminishes and finally attains its initial rate after one hour. The absorption of lead is diminished by the addition of calcium to the diet (Lederer and Bing, 1940), but is not affected by the addition of phosphate ions. Soluble arsenates (Fairhall, Miller and Weaver, 1943) markedly decrease the storage of lead both in

the bones and the kidneys possibly either by decreasing absorption or increasing excretion

The anemia characteristic of lead poisoning is considered by Maugeri (1940) and also by Preti (1938) to be exclusively due to hemolysis. Duesberg (1931) considers that the origin of lead anemia is related to destruction of the red blood cells as well as injury to the bone marrow. Lourau and her associates (1939, 1942) have shown that various liver extract preparations all of which were known to be useful in the treatment of pernicious anemia produced corpuscle regeneration when injected into rabbits made anemic by administration of neutral lead acetate.

While the basophilic granulation or stippling of the red blood cells is somewhat limited as a diagnostic sign in lead poisoning, it is none the less most useful to an experienced observer. Although it is well known that individuals who have suffered prolonged exposure to lead may exhibit little or no stippling, this basophilic granulation of the red cells when considered with other factors is a valuable index of lead absorption. Belknap (1940) considers that more than 12 stippled cells per 50 fields examined in the face of known lead exposure, indicates abnormal lead absorption.

It has been pointed out by Henning and Keilhack (1940) that a much higher percentage of erythrocytes showing basophilic granulation is regularly found in the bone marrow than in the blood in lead poisoning—as high as a ratio of 9:1 in one case. The erythrocytes are present in the sternal puncture even when they are absent in the periphery according to Bensath and Varga (1940) who draw attention to the diagnostic importance of this examination.

For a number of years attention has been directed to a possible porphyrin index as an indication of the extent of lead absorption. Both blood and urine have been examined from this point of view. In cases of lead poisoning, Vighani (1935, 1936, 1937, 1938) finds porphyrin appearing in urine as well as in the blood and feces. The normal urinary level ranges from 10 to 50 micrograms in a twenty four hour sample of urine, while quantities as high as 1 to 3 mgm were found in new cases with clinical manifestations of lead poisoning. In one case the intravenous injection of 25 mgm of colloidal lead was followed by a porphyrin elevation within 48 hours. Carrié (1936), Matuda (1939), Schafer (1938, 1939), Roth (1938), Putnoky and Sumegi (1938), Otto (1938), Vannotti (1938), Meyer (1939), Cappellini (1939), Binet, Parel and Glotz (1939) and Huneki (1940) have variously called attention to porphyrin excretion as a factor of diagnostic importance in lead poisoning. In general these investigators found porphyrinuria following lead absorption. However, this is not specific for lead, since porphyrinuria has also been found in poisoning with arsenious acid, cresol and benzene (Huneki, 1940). On the other hand, Gabel (1940, 1941) and Bjorkman (1941) found no definite increase in porphyrin excretion related to lead poisoning. Lead poisoning produced by feeding lead salts or the inhalation of lead dusts increases urine porphyrin at times but according to Gabel is not a reliable sign of lead poisoning. In view of the fact that increased urinary porphyrin excretion occurs following ingestion of a number of toxic substances as



well as such drugs as sulphonol, it would appear that porphyrinuria as a diagnostic sign in lead poisoning should be treated with some reserve

Because of its unique properties mercury has long been used in industry and poisoning from it has been well recognized. An estimate of the number of workers in the United States actually or potentially exposed to this hazard (Bloomfield, Trasko, Sayers, Page and Peyton) was 32,855 in 1940. With its spreading use in the arts and manufactures, its early use in the silvering of mirrors, in the manufacture of thermometers and barometers, in gilding and in a variety of other ways, the pronounced ill health associated with its use has long been a source of comment among those interested in industrial diseases. The distinctive tremor, loss of teeth, fetid breath and salivation are familiar characteristics of mercury workers who have suffered undue exposure to this substance.

Poisoning by mercury, where it is not acute, is slow and insidious and while chronic mercurial poisoning does not lead to rapid death, its impairment of tissue functions is so drastic that the worker is in a worse plight than with many of the other industrial poisons. Mercury is constantly giving off vapor and thus it contaminates the air where it is exposed. Thus, at 20°C the concentration of mercury in air saturated with mercury vapor is 1.84 parts per million, while at 40°C the concentration rises to 8.5 parts per million. The degree of atmospheric saturation with mercury vapor depends upon the temperature, pressure, rate of air exchange and amount of surface exposed. Stock (1934) has proposed the use of activated charcoal impregnated with about 5 per cent of iodine as a measure of protection against mercury vapor and suggests the use of this material in gas masks. He has advocated the use of this preparation sprinkled upon open areas or cracks contaminated with metallic mercury as a means of fixing the mercury vapor and thus preventing its further distribution.

Stock (1943) estimates the normal daily urinary excretion of mercury to be 0.1 to 1.0 microgram and states that this may rise to several milligrams in persons with amalgam dental fillings or who are in industrial contact with mercury.

While mercurialism as an occupational disease has disappeared in certain trades, such as the silvering of mirrors, new uses appear from time to time such as in connection with the manufacture of radio tubes of a certain type, or in the operation of mercury boilers, or in shooting galleries.

The increased use of cadmium in industry has largely resulted from its use in electroplating. Somewhat parallel with this increase there has been an increased amount of poisoning. In general the latter has not been primarily due to the electroplating process, but to the subsequent firing or welding of cadmium plated material or the over-heating and oxidation of cadmium metal. Cadmium is but little used as a metal directly. It achieves its greatest importance as a constituent of alloys and in the form of its compounds. In this way it is used in bearing metals, electrical conductors, pigments, ceramics, in process engraving, cadmium vapor lamps and for rust-proofing tools, wires and other iron and steel articles, particularly marine hardware and other fittings, which were formerly zinc coated.

A rather large increase in the number of cases of cadmium poisoning—mostly

following ingestion—has occurred since 1941. Prior to 1941 a total of 20 cases of cadmium poisoning due to the ingestion of cadmium had been reported in the literature. However since January 1941, 268 cases of cadmium poisoning following ingestion have been reported (Cangelosi, 1941, Frant and Kleeman, 1941). The majority of these cases occurred as a result of using cadmium coated containers for food or drink. Largely as a result of this the Sanitary Code of New York City was amended to prohibit the use of cadmium in articles used in the preparation or storage of food or drink (New York City, Sanitary Code, Section 145).

The symptoms of cadmium poisoning produced by ingestion are increased salivation, choking attacks, persistent vomiting, abdominal pain, diarrhea and tenesmus (Lewin, 1929). Most often fatalities from cadmium poisoning have resulted from inhalation of the fumes of cadmium oxide, however, rather than the ingestion of cadmium salts. The first symptoms of industrial cadmium poisoning are usually dryness of the throat, cough, headache, vomiting and a sense of constriction of the chest. The later symptoms are referable principally to the respiratory system and are characterized by cough, pain in the chest, severe dysphoria and prostration. These symptoms result from a pneumonitis which in many instances is followed by broncho-pneumonia. Unfortunately, cadmium oxide fumes have no pronounced odor nor immediate irritant effect and can be breathed in fatal concentration without enough discomfort to drive the worker away from the exposure.

In view of the extent and severity of cadmium poisoning in industry a concerted educational effort has been proposed to apprise both manufacturers and users of the hazards involved in the use of cadmium coated products and safety regulations have been proposed defining the degree of maximal permissible exposure of industrial workers (American Standards Association, 1941).

Industrial manganese poisoning is relatively rare considering the manifold technical applications of its compounds. The literature reveals a total of only 353 cases of manganese poisoning which have occurred since 1837, when Couper first reported five workers affected by this disease while employed in the grinding of manganese ore which was once employed for the manufacture of chlorine gas by the old Deacon process. Industrial manganese poisoning in general occurs through the absorption of fumes or dust through the respiratory system following the inhalation of manganese ore dust, or fumes from metallurgical processes—particularly the manufacture of manganese alloy steel. The mining of pyrolu site, grinding, sorting and loading manganese ores have all contributed cases of manganese poisoning as well as smelting operations. Manganese poisoning has been reported in electric welding by workers using electrodes containing manganese (E. Beutker, 1932). Elstad (1939) has reported that following the introduction of an electrical plant for manganese smelting in Sauda in Norway there was a tenfold increase in fatal croupous pneumonia in that region. This was associated with the dissemination of vapors rich in manganese oxide, particles of manganese of less than 5 microns in size being demonstrable in the atmosphere.

Exposure to manganese-containing dust for as short a period of time as 3

months may be sufficient to cause symptoms of manganese poisoning but the usual history indicates an occupation of from one to three years in this dusty trade. The early symptoms of poisoning include languor and sleepiness which precede a complaint of weakness in the legs. A stolid mask-like facies develops. Marked emotional disturbances such as uncontrollable laughter or weeping occur and a peculiar stiffness of the muscles is apparent which results in a spastic gait and in propulsion and retropulsion. Occasionally a peculiar gait, the so-called cock walk (von Jaksch) is observed (Charles, 1927). On the whole the clinical and anatomical picture of manganese poisoning points to involvement of the basal ganglia (Voss, 1939). Well established manganese poisoning is a crippling disease with permanent disability particularly with regard to the use of the legs. Early cases, however, recover spontaneously if the individual is placed in an environment free from manganese dust. The importance of early recognition of manganese poisoning is therefore evident.

The exposure in industry necessary to produce manganese poisoning is usually gross and prevention can be accomplished by the installation of relatively simple mechanical measures, such as are usual in dust control.

The outstanding metallurgical development of beryllium within the past five years and the importance of beryllium alloy for war uses has focused attention upon this very important metal. In practice, beryllium is used in alloy form rather than as the pure metal. The alloys of beryllium and copper represent its most important use at the present time (Sawyer, 1940), although aluminum and nickel both form alloys with beryllium having such singular properties that further development and use of these alloys in industry is assured. The presence of the small amount of beryllium (usually less than 3 per cent) profoundly affects the mechanical properties of the metal. The copper alloy has astonishing fatigue resistance. Springs made from this substance retain their elasticity almost indefinitely even in atmospheres highly corrosive to steel springs. Beryllium copper is used in the delicate mechanism of airplane instruments and in gasoline and oil pipe lines in airplanes where vibration is excessive.

Gelman (1936, 1938) has drawn attention to the severe poisoning of workers engaged in the extraction of beryllium by means of the electrolytic reduction of its fluoride salts. Although the earlier investigation of Weber and Engelhardt (1933) indicated that the cause of disease in beryllium plants is due to the fluorides occurring in the form of dust, Fabroni (1935) regarded beryllium carbonate itself as the causative agent.

A recent investigation of beryllium in this laboratory (Hyslop et al., 1943) has shown that the beryllium ion itself is relatively non-toxic. No consistent pathological change can be attributed to beryllium. Whatever toxicity has been found to occur with exposure to beryllium salts is due to the toxicity of the acid radical such as the fluoride or oxyfluoride, or to an objectionable condition brought about by the hydrolysis of certain of its salts, such as the chlorides or sulphates following inhalation. Owing to the ease and extent to which beryllium salts hydrolyze, the inhalation of dust or fumes of these substances results in pronounced local irritation. Since no safe operating standards should be based

upon the metal itself, consideration should be given to the acid radical with which it is combined, or to other factors, such as the ease of hydrolysis of its salts.

Certain special steels of great importance for war material and having extensive industrial application contain molybdenum. This silver white metal is malleable and can be forged at red heat, but fuses only in the arc. The most useful form is as an alloy of iron with or without association of other metals—the so-called molybdenum steel. Molybdenum steel is used in modern armour plate, armour-piercing shells, rifle linings and high pressure boiler plates. Owing to its extreme hardness and resistance to many corrosive chemicals these steels obviously have also many other important uses.

Exposure in industry occurs in certain dusty operations such as crushing and milling molybdenum ore, heating and rabbling the ore to form calcium molybdate—the form in which it is added to the charge in steel blast furnaces—and in rolling red hot billets of molybdenum steel which give off fumes of molybdenum oxide owing to superficial oxidation. While exposure of workers to dust and fumes of this type has raised the question of possible toxic effects, no comprehensive study has been reported with reference to the industrial toxicology of molybdenum. Pharmacological studies with reference to ingestion (Karantassus, 1924, Franke and Moxon, 1935, Mukherjee, 1936, Ferguson, Lewis and Watson, 1938, Teresi, Elvehjem and Hart, 1942) and to injection of molybdenum compounds (Lampe and Klose, 1912, Agnoli, 1932, Caujolle and Roche, 1935, Anon 1937, Marish, Lustock and Cohen, 1940) have in general indicated a toxic effect less than that of tungsten compounds. Experimental work now in progress in this laboratory particularly with reference to the possible injurious effect of inhalation of the dust of molybdenum compounds or of molybdic oxide fumes has shown that the order of toxicity of molybdenum is not particularly high.

Previous to the closing of the Burma Road we relied upon China as a source of supply of tungsten ore. Tungsten is used in the preparation of high-speed steels, in cemented tungsten carbides and in electric light and radio tube filaments. Cemented tungsten carbide is used as an abrasive containing tungsten, titanium and carbon. These abrasives are long-lived, are extremely hard and are therefore important in industry. The high-speed tungsten steels maintain a sharp cutting edge in lathe tools even when the tool becomes red hot in machining operations. The tungsten carbide alloy is a substance which perhaps ranks next to the diamond in hardness. It is being produced in large quantities for special tips to tools used in machine operations and it is claimed that one man with tungsten steel tools can produce as much as five men with carbon steel tools.

Exposure to tungsten in industry is related chiefly to the dust arising from crushing and milling of the two chief ores—scheelite and wolframite. The preparation and use of powdered tungsten in "powder metallurgy" and the preparation of cemented tungsten carbide tool tips necessarily cause some exposure to tungsten dust. Exposure to tungstic oxide dust also occurs in the production and drying of the latter. Although very little has been published with reference to the toxicity of tungsten in general, Karantassus (1924) regards tungsten as more toxic than molybdenum on the basis of ingestion and injection

experiments Pulewka (1935) has investigated the physiological action of tungstates and has concluded that with warm blooded animals the cause of death is respiratory paralysis resulting either from the toxic effect on the respiratory center or from circulatory disturbances

Kinard and Van de Erve (1941) found ammonium paratungstate upon ingestion to be much less toxic than either tungstic oxide or sodium tungstate Experimental work is in progress in this laboratory with reference to maximal permissible aerial concentration of tungsten dusts to which workers may be exposed

Although antimony and arsenic are usually regarded as having comparable toxic qualities, from an industrial point of view the toxic effects of arsenic outweigh those of antimony Arsine, for instance, has been the cause of many fatalities in industry, but a search through the literature has failed to disclose a clear-cut fatal case of stibine poisoning in man Where stibine poisoning has been reported it has usually been questionable whether the poisoning might not have been due either to arsine, hydrogen sulphide or phosphine

While arsine can be readily produced along with hydrogen by the reaction of a metal such as zinc or magnesium with an acid solution of an arsenical salt, stibine is similarly produced with more difficulty Furthermore, stibine does not have the stability of arsine and is easily decomposed by a variety of substances, such as oxygen, moisture and rubber The instability of stibine, whether prepared by electrolysis or by the action of acids on antimony alloys, makes it difficult to obtain a sufficiently high concentration of stibine to study its effect on large animals (S H Webster, 1944)

While stibine is not easily formed, Hunter (1932) has shown that even quenching material containing arsenides with water has caused arsenical poisoning and deaths Arsenic poisoning has also been reported by Jötten (1942) from quenching iron alloys with water, where the alloy contained arsenic as an impurity More recently similar cases were reported by Nau, Anderson and Cone (1944) and by Dernehl, Stead and Nau (1944), although the question was raised in this latter instance as to the probability of stibine or hydrogen sulphide poisoning

Glaister (1908) reported a total of 120 cases of arsine poisoning to that date and fatalities have since been reported from time to time Cases of poisoning occur in pickling metals where arsenic is present either in the acid or in the metal, in cleaning acid storage tanks where washing has been incomplete, or in certain electrolytic processes Arsine gas from submarine storage batteries was said to have caused poisoning aboard submarines during the last war (Giordano, 1916, S F Dudley, 1919)

The action of arsenic salts or compounds in general is different from that of arsine The latter causes vomiting in most cases, albuminuria, jaundice and produces a hemolytic effect on the red blood cells Fatal termination is frequent Arsenical dusts, on the other hand, usually produce troublesome skin lesions—first of all an edematous condition with inflammation and eventually ulceration Perforation of the nasal septum is common Slow absorption does occur however and is evidenced by bronzing, while in severe cases gastric symptoms occur

Antimonial poisoning on the other hand does not appear to be an industrial disease of any consequence. Rambousek (1913) stated that "it seems doubtful if industrial poisoning can really be traced to antimony or its compounds, generally the arsenic present with the antimony is at fault." Oliver (1933) in an investigation of the health of antimony oxide workers concluded that although the occupation of these men involves exposure to air borne antimony oxide—often in considerable amount—the occupation is not unhealthy. Schwartz (1939) could find no cases of dermatitis occurring in a plant where antimony compounds are manufactured.

In spite of the marked difference noted above with reference to the health of arsenic and of antimony workers, it should be pointed out that the soluble compounds of antimony are decidedly toxic. In an occupation where workers suffer exposure to salts of the latter type, suitable protection should be made available.

An interesting development of recent years has been that of the use of the rare metal indium in industry. This metal which retailed for fifteen dollars a gram as late as 1936 has considerably lessened in price as its industrial application has increased. Among its applications in industry is its use in electro-plating cadmium and similar alloy bearings for airplane and other high-duty internal combustion engines (Taylor, 1941). While indium is regarded as a rare metal, it is estimated that several thousand tons could be recovered annually as by-products of the zinc and coal industries (Latimer and Hildebrand, 1940). Added to silver, whether solid or plated ware, it renders it tarnish proof. In general the amounts of indium required for its various industrial uses are small.

Indium is a silver-white ductile metal softer than lead, oxidizable only with difficulty. Owing to its use in plating surgical instruments and many other proposed uses particularly arising from war conditions the question of its toxicity has taken on increased significance. The importance of indium in industry is shown by the bibliographies of Ludwick (1934-40 and 1941-42) which total 58 pages of references and abstracts. In spite of the current industrial interest in indium relatively few investigations concerning its toxicity have been reported. von Oettingen (1932) found the minimum fatal subcutaneous dose for mice to be about 0.06 gram indium citrate per kgm., but daily feeding of a total of 24 grams of indium hydroxide per kgm. for 2 weeks was without apparent toxic effect. More recently McCord, Meek, Harrold and Heussner (1942) found the metal and its salts to have no skin irritant properties but subcutaneous or intravenous injection of indium sulphate or indium chloride indicated that indium salts are toxic when administered in this way. However, no occupational ill health has so far been associated with the use of indium in industry.

Although the toxicity of indium is relatively of slight importance from the point of view of industrial exposure, this is not true of certain other rare metals. Outstanding among these is uranium, a metal which, apart from its industrial application, has caught the popular fancy because uranium 235 holds the possibility of a future source of atomic power.

Since uranium is generally extracted from its ores by wet processes, exposure to dust fortunately is not so serious as in other metallurgical processes. How-

ever, the preparation of alloys from urano-uranic oxide by the aluminothermic process and electrolysis of the fused double chloride,  $K_2UCl_6$ , as well as the manufacture of steel alloys entail a certain amount of exposure to uranium—containing dust and fumes. Uranium is employed in steel to increase its hardness and tensile strength in gun barrels. It is used in the form of sodium uranate for coloring glass a fluorescent canary yellow and as the black oxide for red and black coloration in the ceramic industry. Certain uranium compounds find application in industry as catalytic agents. Uranium dioxide has a new and important industrial application in connection with elimination of the effect of current surges in powerful projection lamps (Dunkel, 1937).

Uranium and its salts are highly toxic. The absorption of small amounts over long periods of time causes a chronic nephritis first noted by Leconte in 1854 following the administration of uranium. Numerous studies of uranium nephritis have since been reported (MacNider, 1911, 1919, Garnier, Schulmann and Marek, 1928, Dominquez, 1928, Mauriac, 1930, Garnier and Marek, 1933, Mauriac and Sernantie, 1933, Binet and Marek, 1934). MacNider (1936) has shown that in dogs poisoned with uranium, hepatic degeneration occurs similar to that seen with mercury poisoning and parallel with the acidosis rather than the dose. Animals surviving a very severe type of hepatic injury not infrequently repair the injury and acquire a high degree of resistance against secondary intoxication of uranium.

Although uranium poisoning in man is somewhat rare, De Laet (1925) reported four workers who were affected while handling uranium salts over a period of about twenty months.

It is fortunate that in view of its toxicity, uranium can be detected in such extremely minute amounts. Since uranium is a radioactive element, it can be detected by means of a sensitive Geiger-Mueller counter in amounts as small as  $10^{-12}$  grams (DeMent and Dake, 1941).

Magnesium and zirconium fall into a different category with reference to industrial exposure. The former is now being manufactured in enormous quantities and is a valuable and necessary constituent for various light metal alloys. The latter, although formerly of some importance as the oxide in refractories, has become especially useful in tracer bullets, flares, flash bulbs and detonators. Although the physiological properties of magnesium salts are well known it is comparatively only recently that attention has been drawn to injuries in industry from magnesium apart from burns. A few years ago attention was directed to a peculiar form of disease in workers handling magnesium or magnesium-aluminum alloys (Gissel, 1936, Gerlach, 1936, Ehrlich, 1936). This disease results from particulate magnesium or magnesium alloy introduced as splinters, cutting chips, slivers, or turnings into cuts or wounds. Following this, evolution of gas occurs, usually accompanied by local tissue necrosis and occasional gangrene limited to the locality of the gas tumor.

Investigation of tissue reaction to magnesium by McCord, Prendergast, Meek and Harrold (1942) has shown that macroscopic gas tumors may be induced by the implantation of particulate magnesium and high magnesium content alloys.

As little as 10 mgm of powdered magnesium is capable of producing macroscopic tumor masses. In puncture wounds caused by magnesium or magnesium alloys the necessity of removing all the magnesium is apparent, since this may lead to a more severe type of injury than the usual ordinary foreign body injury.

Following the earlier work, a tendency has developed to refer to magnesium injury as "magnesium poisoning" or "duralumin poisoning"—a tendency which is to be deplored as no one has established magnesium as toxic in the usual sense (Schützeberg, 1937, Technische Rundschau, 1941).

According to Gay (1942) magnesium and its alloys are—apart from their inflammable nature—among the most innocuous materials with which workmen come into contact. While injury of the above type has been reported in Germany, Gay reports that in his experience over a period of five years in the magnesium industry, which includes experience with men with approximately one and one-half million work days, no time loss has occurred from injury from magnesium splinters.

Zirconium has only a mild pharmacological action and may even lack physiological effect in small amounts. In the form of the soluble tartrate complex, the intravenous introduction of this salt in relatively large doses (0.15 gram/kgm) in rabbits causes death (Lendle, 1934). The zirconium compounds in technical use are largely very insoluble however and no case of systemic poisoning has so far been reported from this source.

Exposure to acid anhydrides, chiefly in the form of fumes or mist, is common in the arts and manufactures. In general, dilution or protective devices against breathing these fumes are sufficient to guard against the corrosive or irritant action of the common acid anhydrides, such as hydrochloric acid vapor, sulphur trioxide or phosphoric anhydride. Certain other acids, such as hydrofluoric acid or hydrocyanic acid are directly toxic. Such substances as hydrogen sulphide, selenium hydride, hydrogen phosphide, carbon monoxide, chlorine, sulphur dioxide, and nickel carbonyl are frequently encountered in industry and require efficient cartridge type masks, or preferably positive pressure (hose) masks for protection of the worker. In special cases, oxygen breathing equipment is necessary.

Because of the great expansion of the light metal industry, the fluorides both as salt fumes and as hydrofluoric acid vapor have become increasingly of interest to the hygienist.

Both selenium and tellurium are increasing in use the latter chiefly for hardening and toughening of metals and to increase the toughness and resistance to abrasion of rubber hose and cable coverings. Selenium is used in the glass and ceramic industry and to a less extent to improve the machinability of certain steels and copper base alloys. Dudley (1938) has reported pallor, gastrointestinal disturbances, garlicky breath and nervousness in men employed in copper refineries engaged in extracting or purifying selenium. While the bad effects of selenium in soils have perhaps been exaggerated (Lakin, Williams and Byers, 1938), the toxicity of hydrogen selenide is high and the use of this substance requires careful control in industry. In man a concentration of 0.005



mgm per liter is intolerable producing eye and nasal irritation (H C Dudley, 1941)

Interest in fluorine and its compounds has been growing not only because of the great expansion of aluminum and magnesium production since the war, but also because of the increased use of cryolite sprays in orchards to prevent the ravages of the codling moth. Popular interest has centered in the fluoride content of drinking water because of its rôle in causing mottled teeth and also because of its apparent relation to dental caries (Dean, Jay, Arnold, McClure and Elvove, 1939). Finally enormous quantities of hydrofluoric acid are now demanded by the petroleum industry as a catalytic agent. In the presence of hydrofluoric acid and certain other agents, paraffins will add to olefins bringing about certain molecular rearrangements and yielding highly branched products which have good anti-knock value (Ipatiev, 1935). Fumes from arc welding operations, where the welding rods are fluoride-coated to aid in fluxing, are a potential health hazard (Brandt, 1943).

The soluble fluorides act as protoplasmic poisons (Greenwood, 1940). The soluble neutral salts destroy the cells of mucous membranes, while the acid causes deep tissue destruction and slow healing burns which probably result from its specific toxic action. On inhalation Machle, Thamman, Kitzmiller and Cholak (1934) showed that exposure to concentrations of hydrogen fluoride above 1.5 mgm per liter for any period of time is dangerous for rabbits and guinea pigs, while concentrations below 0.1 mgm per liter were tolerated for 5 hours without injury sufficiently severe to cause death.

According to Greenwood (1940) pathological changes which have been reported in organs and tissues as characteristic of fluoride intoxication should be accepted cautiously. However, some histological changes were noted (Biester, Greenwood and Nelson, 1936) in the small intestine, spleen, urinary bladder, mesenteric lymph nodes and thyroid gland of dogs fed fluoride at levels of 0.45 to 4.52 per kilo of body weight. In general, there appears to be some variation in the toxicity of different fluorine compounds and in the sensitivity of various species to the same fluorine compound.

Exposure to fluoride fumes in toxic concentrations usually manifests itself by a sudden onset of chills followed by a symptomless period lasting one or two days or longer. A second phase follows characterized by shortness of breath, a cough with scanty secretion, dyspnea and elevated temperature. Cyanosis becomes apparent. The blood shows a leucocytosis and the sedimentation rate is increased. All these symptoms subside gradually. The duration of the process varies from ten days to one or two months. Clinically the pulmonary process is similar to bronchiolitis (Roholm, 1937).

While the comprehensive report of Roholm (1937) on the effects of exposure of workers to cryolite dust has pointed out the severe bone changes resulting from this exposure, no similar study has been reported involving osteosclerosis resulting from exposure to hydrofluoric acid.

Chromic acid anhydride and vanadic acid anhydride both cause occupational ill health. The use of the former in chromium plating baths as well as in those

industries where exposure to the dust of chromates occurs, gives rise to dermatitis, chrome ulcers and perforation of the nasal septum. Although used in dissolved form in the plating bath the evolution of gas from the electrodes carries mist and spray into the air necessitating the use of careful ventilation in order to reduce this hazard. The derivatives of hexavalent chromium are the only compounds of industrial toxicological interest (Akatsuka and Fairhall, 1934) since trivalent chromium derivatives seem more or less physiologically inert.

While systemic poisoning from chromates is rare, the unpleasant effects of exposure to chromate dust or mist where environmental control is inadequate leads to a large labor turn-over.

A new source of chromate exposure was recently shown to occur by Greenburg and his associates (1942) with reference to spray painting. In a survey of 106 painters in a large airplane factory in New York State several cases of perforated septums were found which were attributed to the large amount of zinc chromate pigment present in the paint.

At the present time magnesium castings and light metal die castings are frequently treated with a solution of sodium or potassium dichromate. Steam issues from the solution carrying the chromate salt which is deposited as a yellowish solid on all near-by surrounding objects (Hypher, 1941). Workmen are frequently afflicted with dermatitis from this source, with perforation of the nasal septum and where splashing of the liquid occur in contact with skin abrasions indolent "chrome sores" or "chrome holes" occur. It is usually impossible to cure this condition while the sufferers continue in their usual employment.

While chromium plating has given rise to much occupational ill health as a result of the popular demand for chromium plated articles and the burgeoning of this industry as a consequence, a newly devised process will likely afford relief in this direction. The new Warner (1944) method uses trivalent chromium salts which not only promise to revolutionize chromium plating itself in a technical sense, but, since these salts are non toxic, should eliminate the chromic acid hazard.

Most of the accounts of industrial vanadium poisoning refer to the observation of Dutton (1911), which are general in tone. Shortly after this was published, Lees (1911) took exception to the statements of Dutton with reference to the extent of vanadium poisoning in industry. However, the most illuminating discussion of industrial vanadium poisoning to date is that of Symanski (1939) who made a careful study of nineteen cases of vanadium poisoning amongst metallurgical employees in Germany. Although the bulk of vanadium is used for metallurgical purposes, it also is extensively used in many other unrelated industries. One of its most useful applications is as a catalytic agent for certain chemical processes. In general, it is used as an oxidizing catalyst—for instance, for converting naphthalene into phthalic anhydride, anthracene into anthraquinone, toluene into benzaldehyde and benzoic acid (Alexander, 1929). In the catalytic conversion of sulphur dioxide to sulphur trioxide, it is far less expensive than platinum, and has the advantage that it is not poisoned by arsenic or

hydrogen chloride Furthermore it steadily converts 97 to 99 per cent of the material Nickell (1928) stated that it is likely that vanadium catalysts will eventually completely replace platinum in the catalytic manufacture of sulphuric acid

Although many therapeutic applications of vanadium salts have been made, the usefulness of vanadium in the treatment of disease is questionable Minor industrial applications are to be found in dyeing, in glass manufacture and as a dryer in paints and varnishes Although by far the greatest application of vanadium in industry is to be found in the metallurgical field, these minor applications in the aggregate represent a sizable potential health hazard The vanadium alloy steels (Saklatwalla, 1920) being especially hard and having high fatigue resistance, have a wide variety of application such as in tools, in crank shafts for automobile and Diesel engines, in the spring leaves and rear axles of motor cars The nonferrous alloys also are very hard and are resistant to atmospheric corrosion The importance of vanadium in industry is indicated by the great increase in imports and consumption previous to the war The consumption of vanadium for instance for 1937 more than trebled that of 1936 (Ridgway and Davis, 1938, 1941) while the world consumption more than trebled between 1936 and 1939 This increasing consumption implies the possibility of an increased industrial exposure

While the physiological effects of the salts of vanadium have long been known (Priestley, 1876, Gamgee and Larmuth, 1876, Larmuth, 1876, Dowdeswell, 1878) interest in its toxic action has largely been confined to pharmacological studies (Luzzatto, 1903, Ricciardi, 1910, Jackson, 1912, Manz, 1913, Proescher, Seil and Stillians, 1917, Heimberger, 1929, Ballotta, 1931, Roffo, Calcagno and Ramirez, 1932, Franke and Moxon, 1936, Daniel and Lillie, 1938, Cavalli, 1939, Molland, 1940) and very little cognizance has been taken of its importance as a cause of occupational disease

It is of interest that, in spite of its pronounced toxicity, the number of cases of vanadium poisoning in industry has been relatively small It is probable that one reason for this is to be found in the method of extraction Since only small deposits have been found outside the Peruvian and South African sources, vanadium-poor countries have extracted vanadium from certain ores and minerals, coal ashes, asphalt and petroleum residues Most of these procedures are long and complicated and largely carried out in the wet way In handling the Peruvian ore—vanadium sulphide or patronite—a certain amount of exposure occurs in milling and mixing the furnace charge It appears to be the case both in this country and in Germany (Symanski, 1939) that only the workers themselves seem to be aware of the effect of exposure to vanadium on health Symanski in his review of nineteen cases of vanadium poisoning points out the acutely irritant effect of vanadium compounds The characteristic effect is a chronic bronchitis which, with continued exposure, may result in specific infectious complications Symanski recommends the mechanization and enclosure of all work processes which are dusty in nature

Silicosis is one of the oldest of occupational diseases and yet it has received

extended investigation only within the last two decades. This disease, wholly dust-borne, arises from the inhalation of particulate matter containing free or uncombined silica. It is characterized anatomically by generalized fibrotic changes with miliary nodulation in the lungs. Clinical signs are shortness of breath, a lowered vital capacity, a lowered capacity for work, increased susceptibility to tuberculosis and a characteristic x-ray appearance of the lungs.

This disabling disease is found amongst workers engaged in mining, tunnelling, stone cutting (granite), metal grinding, foundry and blasting and scores of occupations where the worker is continuously exposed to a dusty atmosphere containing particles of quartz or free silica. Lanza (1934) conservatively estimated the number of workers exposed to dangerous amounts of silica dust at 500,000 in this country. A later estimate (Bloomfield et al., 1940) places the number at more than one million.

The disabling nature of silicosis, the extent of exposure in industry and the number of workers involved have naturally motivated many investigations of its cause, pathology, complications and prognosis as well as its allied forms, silico-tuberculosis, anthracosis and asbestosis (Mavrogordato, 1918, Pancoast and Pendergrass, 1926, Kettle and Hilton, 1932, Gardner and Cummings, 1933, Miller and Sayers, 1934, Policard, 1934, Robson, Irwin and King, 1934, Giese, 1935, 1936, McCord, Ainslee, Johnston and Fleming, 1936, Briscoe, Mathews, Holt and Sanderson, 1937, Gardner, 1937, Denny, Robson and Irwin, 1937, 1939, Stoeckel, 1937, Hollmann, 1937, Weiland, 1938, Lambie, 1938, Irwin and Gibson, 1938, Naeslund, 1940, Baader, 1941, Zech, 1942), while a number of field studies of large groups of workers (Winslow and Greenburg, 1920, Collis and Gilchrist, 1928, Thompson, Brundage, Russell and Bloomfield, 1928, Russell, Britten, Thompson and Bloomfield, 1929, Fehnel, 1929, Merewether and Price, 1930, Collis and Yule, 1933, Sayers, Meriweather, Lanza and Adams, 1933, Quaintance, 1934, Riddell, 1934, Dreessen and Dallavalle, 1935, Haldane et al., 1935, Middleton, 1936, Sutherland, Mencklejohn and Price, 1937) have added greatly to our knowledge of this occupational hazard.

As a result of the many surveys that have been made of workers and their environment with reference to silicosis, efficient protective measures in general have been set up in the working establishments. Dust control has been instituted both with reference to particle size and to dust concentration. There is more or less general agreement that it is desirable to avoid concentrations of more than 5,000,000 particles per cubic foot of air in working places where the dust contains a high percentage of free silica (Meller, 1937). Granite dust which contains about 35 per cent free silica, when in concentrations of 10,000,000 to 20,000,000 particles per cubic foot has been found not to cause disabling silicosis in a working life time while anthracite dust, containing less than 5 per cent free silica, has been found not to cause anthraco-silicosis in concentrations of less than 50,000,000 particles per cubic foot (Meller, 1937).

Although the matter has been so extensively investigated, the mechanism of action of silica in causing silicosis is still unexplained. The theory of Jones (1933) that the mineral sericite (a hydrated silicate of aluminum and potassium)

is the chief factor in the development of silicosis is no longer tenable (Gerstel, 1938) Numerous experiments have been made with various other dusts in conjunction with free silica in order to find which dusts enhance the effect of particulate silica and which decrease it Certain substances such as aluminum, iron and magnesium dusts when used with quartz, as well as coal and cement dusts, have been found to decrease the pulmonary changes associated with free silica alone Outstanding in this respect is the work of Denny, Robson and Irwin (1937, 1939) who have shown that aluminum dust can bind or inhibit the solution of silica The extent to which aluminum dust can be relied upon to delay or check the development of silicosis is difficult to evaluate at the present time, but it is conceded that this work represents a significant development in silicosis research

In general there is a tendency in industrial hygiene to seek out the level of physiological tolerance and to try to maintain working conditions at that level or at lower concentrations with respect to industrial poisons The present trend in industrial hygiene is in the direction of prevention To this end all-enclosed operations are conducted when possible Great advances have been made in this direction since the last war However, the field is somewhat limited in which this procedure can be applied By their very nature many operations can only be carried out openly with attendant risk of exposure to gases, dusts and fumes In those cases where toxic materials are thus used, it is necessary to protect the workers with masks of a type suitable to the hazard involved These and other safety measures have very materially lessened industrial injury—particularly during the past decade Much still remains to be done, however, especially in smaller establishments where, due to ignorance or because of cost, employees are still subjected to unhygienic working conditions

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## ERRATUM

Volume 25, 1945 Paper by J W Bean Effects of Oxygen at Increased  
 Pressure Page 69, 2nd paragraph, line 14, which reads " $137.5 + (3 \times 360)$   
 $+ 1226.5$ " should read " $137.5 + (3 \times 360) + 9 = 1226.5$ "

# PHYSIOLOGICAL REVIEWS

VOL. 25

APRIL, 1945

No 2

## THE ADRENAL-GONAD RELATIONSHIP

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**I INTRODUCTION** The existence of a functional relationship between the adrenal cortex<sup>1</sup> and the gonads was originally demonstrated by the clinical observation that disturbance of the adrenal glands might result in alteration of sexual functions or characters. In the last decade, however, many experiments on animals have shown that there is a close connection between the two organs, and the gradual working out of the details of the reciprocal inter-relationship has been one of the most interesting phases of the expansion of endocrinological knowledge in recent years.

The evidence at the present time concerning the adrenal-gonad relationship is derived from the study, first, of the effects of the gonads on the adrenals and on the various physiological activities controlled by the adrenals, as shown by functional correlation, gonadectomy experiments and the injection of sex hormones, and secondly, of the effects of the adrenals on the gonads and on the accessory reproductive organs as shown by functional correlation, adrenalectomy experiments and the isolation of gonadal hormones and functionally analogous substances from adrenal cortical extracts.

Changes caused by the gonads in the adrenals and thence in those bodily

<sup>1</sup> Of the two components of the adrenal gland the cortex only is referred to in this article but for convenience the term adrenal gland is used throughout.

activities controlled by the adrenals must be clearly distinguished from direct corticoid activity on the part of the gonad hormones. Similarly, changes produced by the adrenals in the gonads and thence in the accessory organs must be clearly distinguished from direct effects produced on the accessory organs, either by the production in the cortex of androgens, oestrogens or progestogens, or by the gonad-hormone-like activity of the essential cortical hormones themselves.

In the review which follows, subdivision of subject is employed as far as possible to assist orderly presentation of the extremely heterogeneous mass of material. The subdivision cannot be complete, and some overlapping is unavoidable. Thus, discussion of the effects of corticosterone on the reproductive organs and of the presence of gonadal hormones in the adrenals is difficult to separate exactly from discussion of the effects on the reproductive organs of crude adrenal extracts, which may contain both of these substances. However, in the main, the aim has been to make each section self-contained. The literature discussed is that which appeared up to the end of 1941.

II THE EFFECTS OF THE GONADS ON THE ADRENALS (a) *Sex dimorphism in the adrenals* Sex dimorphism of the adrenals is found in a number of species. It usually takes the form of a size difference, which may or may not be associated with obvious histological distinctions. Hill (193) makes the generalisation that the adrenals of the female at birth are heavier than those of the male in primates, carnivores and ungulates. Such a size dimorphism is, of course, accentuated by the fact that the female is usually of lesser body weight than the male. In rare instances the cortex in one sex or other possesses a special zone, either temporarily or permanently. There is as yet no conclusive evidence of sex dimorphism in function, except in some cases of neoplasia in humans, though it might be expected that the special zone which continues to develop in the female mouse, or which appears in pregnancy in certain species, performs some special function.

*Fowl* Some difficulty is experienced in studying the adrenal glands of birds since clean dissection is difficult without undue manipulation, and the intermingling of cortical and medullary tissue complicates histological examination. Latimer (253) failed to find any difference in the weight of the adrenal glands in male and female fowl, but subsequently Sauer and Latimer (338) reported that the female fowl has approximately 30 per cent more cortical tissue, although the total weight of the gland is about the same.

*Mouse* The best known case of sex dimorphism of the adrenals is to be found in the mouse. The whole gland is much larger in the female than in the male, as pointed out by Masui and Tamura (280) who ascribed the difference to the much greater development of the zona reticularis in the female. Moreover, the percentage of cortex is greater, according to Carlson, Gustafsson and Möller (62) the cortex comprises 82 per cent of the gland in the female, and only 67 per cent in the male. A more detailed study of the morphological difference was made by Howard-Miller (210) and by Deanesly (94) who reported that there was a much greater development of the inner cortical zone in the young

mouse from the age of about five weeks onwards. Howard Miller (210) used the term "X zone" for this region, which consists of small darkly pig cells, to distinguish it from the narrower and less sharply defined zona laris common to males and older females. According to Howard (205) of different strains vary greatly in the amount of X zone tissue they develop, and, therefore, in the degree of sex dimorphism. Gersh and Grollman failed to find any evidence that the X zone performed a special function, they regard it rather as a reserve source of cortical hormones.

Jackson (216) described the adrenal of the female rat as being larger than that of the male, and Hatai (185) came to a similar conclusion and held that the difference became greater with increasing body weight. Later, (186) recorded that the adrenals of the rat, up to a body weight of about 100 gms, are of about equal size in the two sexes. With increasing body weight sex dimorphism appears, and at 300 grams body weight the adrenals are about twice as large in the female albino rat as in the male. Donaldson (106) gives curves which show that at 100 grams body weight the adrenal of the female rat is about one third heavier than that of the male, while at 300 grams body weight the gland of the female is more than 50 per cent heavier. Donaldson (107) also studied the relative contribution of cortex and medulla to this sex dimorphism. King and Donaldson (221) found that in relation to body weight the weight of the adrenal of the female rat exceeded that of the male by 50 per cent. The difference was only about half as great in captive gray Norway rats.

Sex dimorphism in the adrenals of the rat appears to have little histological basis. According to Howard (205) the "juvenile" adrenal of the rat (see p. 206) shows no sex difference. In adults the zona reticularis is somewhat more distinct from the zona fasciculata in the female than in the male.

*Guinea pig.* Deanesly and Rowlands (100) found that the adrenal in the female guinea pig is larger than in the adult male guinea pig. According to Donaldson (115) the adrenals are larger in the male and less variable. Zalesky has recently stated that there is a histological sex difference in the adrenals of guinea pigs, the female having a somewhat wider zona fasciculata and a relatively narrower zona reticularis.

*Rabbit.* Roaf (327) reported that he was unable to find any clear histological sex dimorphism in the adrenals of the rabbit.

Latimer (254) found little difference in the weight of the adrenal glands in male and female cats when reckoned as a percentage of body weight.

Baker (11) noted that the adrenals were larger in dioestrous bitches than in mature males, but that the percentage weights were the reverse. Howard (203) made a detailed examination of the adrenals of male and female *Ateles* monkeys but was unable to find clear histological sex dimorphism. According to Materna (281) and Freeman (140) the adrenal glands in women are slightly larger than those of men, a difference which is presumably obliterated when allowance is made for body weight. Parhon and Zugravu

(303) found the glands of the male to be much larger than those of the female in a series of 224 insane subjects

(b) *Changes in the adrenals associated with the reproductive cycle Pigeon* According to Riddle (325) it is extremely probable that the adrenal glands enlarge considerably at the time of ovulation in the pigeon. The maximum size seems to be attained in the 44-hour interval between ovulation of the first and second eggs. Apart from gross hypertrophy the glands show no unusual features

*Mouse* The X-zone of the mouse develops about equally in the two sexes until the age of five weeks. In the male degeneration then takes place and the zone is replaced by fibrous reticular tissue which forms a capsule round the medulla. In the female this X-zone continues to grow until puberty when it occupies more than half the cortex in the unmated animal. It degenerates gradually in the unmated female and disappears before the end of the reproductive period, the histological changes being the same as in the young male. According to Howard-Miller (210) and Deanesly (94) there are no changes in the adrenal associated with the oestrous cycle of the unmated mouse, though Masui and Tamura (280) had previously reported that hypertrophy of the cortex occurred at oestrus. During pregnancy in the mouse, however, especially in the first pregnancy, there are marked changes in the adrenals. Tamura (374) reported a decrease in the gross size of the adrenal during pregnancy, although there is some hypertrophy of the zona glomerulosa and zona fasciculata, reaching a maximum at the 17 mm stage. The most obvious change, however, occurs between the 7th and 12th days of the first pregnancy when the X-zone undergoes degeneration similar in degree and kind to that which occurs much more slowly in the unmated female.

*Rat* Howard (205) made a detailed examination of the development of the adrenals of young rats and summarises her results as follows: "Rats of both sexes at three weeks of age show a differentiation of the inner third of the cortex, which differs from the adult zona reticularis, and resembles the mouse X-zone in some respects. This inner layer is here termed the juvenile cortex. The juvenile cortex, for the most part, is gradually transformed into the adult reticular zone, and has lost its distinctive character by forty days of age. Small groups of cells of the juvenile cortex may fail to undergo this differentiation, and persist as structurally isolated groups bearing some resemblance to the rat X-zone." Anderson and Kennedy (5) made a detailed study of the adrenal gland during the phases of the oestrous cycle in the unmated rat. They found that the relative and absolute weights of the gland are greater at oestrus than in dioestrus. The oestrus hypertrophy is purely cortical and is caused by enlargement of the cells of the zona fasciculata. Bourne and Zuckerman (25) obtained somewhat similar results in experiments which are described in more detail on p. 230.

During pregnancy, there seems to be but little change in the adrenal of the rat. Herring (189) found that the weight of the adrenal in the non-pregnant rat was 0.2 gram per kgm. body weight, which in pregnancy rose to 0.22 gram

per kgm, an insignificant change. Donaldson (108) was unable to detect any increase in weight during pregnancy and lactation in albino rats free from visible signs of disease. Donaldson (109) did not find any change in the relative volumes of cortex and medulla in the adrenal of the pregnant rat.

*Guinea pig.* Elliot and Tuckett (119) described the post-natal development of the adrenal of the guinea pig and drew attention to the relatively small size of the gland at birth and the great development of the cortex during adolescence. According to these authors the cortex, relative to medulla, is larger in the guinea pig than in any other mammal. Earlier, Guieyresse (170) had described enlargement of the cortex of the guinea pig during pregnancy—a result confirmed afterwards by Kolmer (229), Kolde (228) and Castaldi (67). Guieyresse described the change as being mainly in the cells of the middle of the cortex which increased in size and contained large vacuoles. Kolmer also reported that many mitoses occurred at the time of parturition. According to Verdozzi (387) the hypertrophy continues into lactation. According to Hewitt and Liere (191) the weight of the adrenal of the guinea pig increases relative to body weight in late pregnancy and again post partum.

*Ferret.* It seems probable that the ferret, with a restricted breeding season and marked seasonal changes in the reproductive organs, would show corresponding changes in size in the adrenals, reflecting increase and decrease of hypophyseal activity, but no observations appear so far to have been recorded.

*Rabbit.* Early work on the development of the adrenal cortex of rabbits was carried out by Gottschau (163). Later, no hypertrophy of the cortex during pregnancy in the rabbit was found by Kolde (228) and Stilling (368). According to Randall and Graubard (321), however, there is considerable enlargement of the rabbit adrenal during the second half of pregnancy, together with an absolute increase in the amounts of phospholipid, free and ester cholesterol, neutral fat and total lipid. Only the neutral fat, however, increased in percentage amount.

*Squirrel.* Seasonal changes in the adrenal of the thirteen lined ground squirrel have been described by Foster (135) who noted that the gland was small and poorly vascularised in anoestrus, the histological picture being one of inactivity. As the breeding season approaches, the glands, along with the gonads, increase in size by cellular proliferation and increased vascularity. The hypertrophy lasts throughout oestrus and pregnancy but involution starts shortly after parturition. Zalesky (399) also found that a significant increase in the size and weight of the adrenals, due to cortical hypertrophy, takes place in both male and female animals during the breeding season or when the gonads are artificially stimulated during anoestrus. Histologically he found that the hypertrophy consisted mainly of expansion and differentiation of the zona reticularis.

*Mole.* Kolmer (230) examined a number of pregnant and non pregnant moles from February to April, at the beginning of the breeding season, and found adrenal enlargement at this time.

*Opossum.* Bourne (23) has described a remarkable hypertrophy of the

adrenal of the Australian opossum (*Trichosurus vulpecula*) during pregnancy. According to this author the cortex in this species cannot be divided into the three typical zones found in other mammals. Completely surrounding the medulla is a thin rim of cortex, divided into an outer fat-containing layer and an inner fat-free layer. These he terms "alpha" and "beta" zones respectively. In the male, both these layers remain thin. In the virgin female the beta zone begins to hypertrophy on one side of the gland and pushes into the medulla, and at maturity its inner cells on the hypertrophied side enlarge by cytoplasmic increase. During pregnancy there is tremendous hypertrophy of the whole of the zone.

*Dog* Baker (12) found a significant hypertrophy of the adrenals during oestrus, but only in certain weight groups. Dioestrus was associated with decrease in the size of the gland. This author concluded that there was no hypertrophy of the adrenal during pregnancy or lactation and only slight hypertrophy during pseudo-pregnancy.

*Sheep* Nahm and McKenzie (293) have described changes in the adrenal during the reproductive cycle in the ewe, the changes mainly consisting of an increase in the number of dark cells, and in the amount of lipid during early oestrus and early and late pregnancy.

*Primates* The relatively enormous size of the human adrenal at birth is well known, according to Hill (193) the fact that the gland at this stage is nearly as large as the kidney was known to Morgagni (1682-1781). The qualitative nature of the phenomenon was elucidated by the work of Starkel and Wegrzynowsky (362), Elliot and Armour (118), Kern (220) and Thomas (375). The gland at birth consists almost entirely of cortical tissue, the great size being due to the development in foetal life of a special inner cortical zone, to which the name foetal cortex is now applied. Immediately after birth this zone undergoes involution accompanied by extensive vascular degeneration and haemorrhage into the tissues. By the end of the second post-natal month the adrenal presents the essential structural features of the adult adrenal, the foetal cortex having given place to the zona reticularis. After the adult type of cortex is established there seem to be no definite changes associated with sex or reproductive condition.

In monkeys, the same pre- and post-natal changes seem to occur as in man. Hill (193) examined various species of macaques, adult and foetal, a foetal lemur and a foetal *Nycticebus*, and came to the conclusion that there was great hypertrophy of the foetal adrenal due to hypertrophy of the deeper cortical layers. This inner cortex, comparable to the foetal cortex of man, undergoes atrophy after birth. Hill notes that its successor, the zona reticularis, is also transitory in male primates. According to Howard (203) there are no changes in the adrenals of monkeys coinciding with puberty analogous to those seen in the mouse.

The adrenals of other mammals do not seem to show anything similar to the post-natal involution of the foetal cortex in primates, but the pre-pubertal degeneration of the "juvenile cortex" of the rat and of the X-zone of the male mouse may represent a similar process occurring at a later stage.

(c) *Effects of gonadectomy* There have been many investigations of the effects of gonadectomy on the adrenals, but much of the work has been based on inadequate series of animals or upon unsuitable criteria. The literature up to 1933 is fully reviewed by Andersen and Kennedy (6) who concluded that much of the older work was of little value. Literature on the effects of ovariectomy on the adrenals was reviewed by Blumenfeld (21). Only the more important of the contributions considered by these authors will be referred to in the present article.

*Fowl* Soli (358) reported that the adrenals of capons were smaller than those of cocks of the same age, but Marrasani and Luciani (274) did not find any regular change.

*Mouse* Altenburger (4) observed that castration increased the thickness of the adrenal cortex of the male mouse, an effect undoubtedly due to the stimulation of the X zone which follows the operation (Masui and Tamura, 280, Howard Miller, 210, Deanealy, 94). This effect is reversed by the grafting of testis tissue into the castrated male (Takewaki, 373). Prepubertal castration causes the adrenal of the male to approximate to that of the female, both in size and histological appearance, owing to the development of a darkly staining X zone around the medulla. If castration is post-pubertal, the adrenal shows a similar reaction, but the X-zone is less well developed. The X zone of the castrated mouse, like that of the female, gradually degenerates with increasing age. Gonadectomy has no effect on the growth or degeneration of the X zone in the female.

*Rat* Hatai (187) recorded extensive experiments on the effects of gonadectomy on the adrenals and concluded that the effects are opposite in the two sexes, there being an increase of size in males and a decrease in females. Andersen and Kennedy (6), however, have criticised Hatai's use of control material and maintain, from a recalculation of his figures, that the results were not so definite as suggested. Minowada (288) found a slight size increase in the adrenal of the castrated male rat, but was unable to detect histological change. Andersen and Kennedy (6) themselves concluded that the degree of the effect was dependent on the sex of the animal, the interval after operation and the age at operation. They found that nine weeks after castration of the immature male the adrenals were hypertrophied, contained more lipoids than the controls and histologically resembled the glands of the immature animal. Castration of the adult male rat produced no size changes and only transient histological ones. Ovariectomy of immature females resulted in the adrenals becoming similar in size to those of dioestrous controls. Ovariectomy of adult females caused a transient hypertrophy, so that the adrenals became similar to those of oestrous controls, followed by atrophy characterised by decrease of lipoid in the zona fasciculata and degenerative changes in the reticularis. A sex difference in response of the adrenals of the rat to gonadectomy was also recorded by Winter and Emery (394) who used sexually mature but not fully grown rats, gonadectomised for three weeks or more, and found hypertrophy of the adrenals in males and hypotrophy in females. Schilling and Laqueur (342) also noted a decrease in adrenal weight following ovariectomy of thyro-hyperplastic rats. The effects



of gonadectomy in rats has also been considered by Korenchevsky and his collaborators Korenchevsky (231) and Korenchevsky and Dennison (236) found hypertrophy in the adrenals of the castrated male, amounting to about 25-40 per cent on a body weight basis Hall and Korenchevsky (175) described this hypertrophy as being associated histologically with increase in width of the zona fasciculata and reticularis and in cell size and vacuolation According to Hall (174) these histological changes, like the size changes, are absent in the adrenals of ovariectomised females

Ellison and Burch (120) using adult females ovariectomised for twenty-five days, also noted atrophy of the adrenals Lauson, Heller and Sevringhaus (255), however, did not find that a 20-day period of ovariectomy had any effect on adrenal weight in adult rats Blumenfield (21) spayed rats shortly after weaning and killed them at three months old, and found that the adrenals were 8.3 per cent less than those of the controls, due to a 9.5 per cent decrease in the cortex For rats killed at six months old the corresponding figures were 33.4 per cent and 35.8 per cent These changes were associated histologically with cell shrinkage and cell destruction Bourne and Zuckerman (25), however, state that cellular enlargement takes place in the zona fasciculata and zona reticularis in spayed rats, and that the adrenals, in the absence of the ovaries, undergo a 5-day cycle of changes similar to that seen in the normal female (Zuckerman, Bourne and Lewes, 410) Hypertrophy of the adrenal of the castrated male rat has also been noted by Chodt (73) and Pinto (313) Howard (205) failed to find regular changes in the histology of the juvenile cortex of the rat following castration, but records one exceptional case of the development of a fairly well defined X-zone in a castrated rat Waterman (390) found average adrenal weights of  $49 \pm 2.1$  mgm in female rats ovariectomised for one week, as compared with a control figure of  $66 \pm 2.6$  mgm Waterman adds the interesting observation that this decrease did not influence the survival time when the rats were subsequently adrenalectomised

These records leave no doubt that the usual response of the adrenals of the rat to gonadectomy is hypertrophy in males and hypotrophy in females These changes go far to abolish the sex dimorphism in adrenal size seen in normal rats

*Guinea pig* One of the more satisfactory of the early experiments on gonadectomy and the adrenals was carried out by Marrassini (273) on male guinea pigs He found a hypertrophy due to vacuolation and increase of fasciculata cell size which was most marked at eight days after operation and was decreasing at 24 days A somewhat similar result was obtained by Rigano-Irrera (326) Lemati (256) observed cortical hypertrophy in each one of a number of young guinea pigs killed from 10 to 80 days after operation Moore (291), however, found that the adrenals were smaller in guinea pigs ovariectomised for long periods A detailed investigation was carried out by Zalesky (400) who gonadectomized guinea pigs prepubertally and killed them approximately 18 months after operation No appreciably permanent hypertrophy was found in either sex The possibility of transient hypertrophy suggested by the work of earlier authors was not investigated The sex differences in the adrenals (see p 205)

persisted in the gonadectomised animals and are therefore either not produced or not maintained by gonadal activity. It may be said therefore that, so far as existing researches go, the guinea pig adrenal may show a transient hypertrophy after gonadectomy, but not a permanent one.

*Rabbit* A number of indifferent researches have been carried out on the rabbit. Hypertrophy of the adrenals after gonadectomy was reported on very meagre evidence by Soli (358), Raineri (320) and Schenk (340), Friedl (144) for females, Kolde (228) and Livingston (263) for males, and Leinati (256) for males and females, and Zchwetadse (402) found some transient hypertrophy. Where histological studies were made the hypertrophy was said to be due to enlargement of the cortex, particularly of the zonae fasciculata and reticularis. On the other hand, Marrassini and Luciani (274) and Dick and Curtis (102) failed to find any effect. The former of these authors provided the most accurate and best controlled material.

*Squirrel* Zalesky (399) found that castration of at least one month duration led to atrophy of the zona reticularis in the adrenal of the thirteen lined ground squirrel.

*Dog* Feodosyeff (129) reported hypertrophy of the adrenals after ovariectomy, but Pianese (312) in a controlled and apparently much more satisfactory experiment was unable to detect any change.

*Sheep, cattle, pig and horse* A rather miscellaneous series of observations on spayed or castrated sheep and cattle were recorded by Marrassini and Luciani (274) but their material does not permit of definite conclusions. Giroud and Santa (156) reported that castration increases adrenal size in pigs, sheep, cows and horses, and also raises the ascorbic acid content.

(d) *Effects of gonadal hormones* The observations recorded above show that there are undoubtedly changes in the adrenals associated with activity or lack of activity on the part of the gonads. If it be assumed that the connection is entirely endocrine it would be expected that the changes could be reproduced or abolished by the injection of gonadal hormones. This expectation has largely been realised, and, in addition, various effects of prolonged heavy dosage have been noted which indicate the possible effects of chronic gonadal hyperactivity. It is uncertain whether the changes are due to direct action of the gonadal hormones or to indirect action exerted through the pituitary body, which is known to undergo changes associated with the reproductive state of the animal and after gonadectomy. Such changes are commonly supposed to relate solely or mainly to the gonadotrophic function of the anterior pituitary body, but the supposition is difficult to prove. Experiments on gonadectomised hypophysectomised animals are complicated by the fact that removal of the pituitary body causes severe hypotrophic changes in the adrenals, compromises their functional integrity and goes far to destroy their capacity to respond to stimuli other than the injection of adrenotrophin. Thus Deane's (95) observation that the X zone does not develop in the pituitary-deficient dwarf mouse, either in the female or in the castrated male, undoubtedly means that the development of the X-zone is dependent on pituitary activity, but it does not show

whether the effect of the testes or injection of androgens in suppressing the development of the zone in the normal animal is brought about by preventing the cortex from responding to pituitary activity or whether the necessary pituitary activity is suppressed at source

It is in the light of considerations such as these that the effects of injections of gonadal hormones on the adrenals must be assessed

*Mouse* Since the development of the X-zone is associated with the absence of the testis, it would be expected that the administration of testis extracts or androgens to the castrated male or the female would prevent the development of the zone or cause it to disappear. Martin (275) failed to find any effect of injecting "testicular hormone" for a few days, but observed that prolonged injections caused disappearance of the zone in castrated males and in females. A similar result was obtained by Poll (314) using an androgenic urine extract. Callow and Deanesly's (59) experiments with small doses of androsterone gave dubious results. Later, however, Deanesly and Parkes (98) obtained unequivocal positive results with 2 to 4 weeks injections into castrated immature mice, of the following substances and daily doses: androgenic urine extract (2.5 I.U.), testosterone (80  $\mu$ g), androstenediol (160  $\mu$ g), and *trans*-androstenediol (250  $\mu$ g). The last of these preparations was interesting in that it inhibited the development of the X-zone, but failed macroscopically to stimulate the accessory reproductive glands. The effectiveness of testosterone and testosterone propionate in suppressing the X-zone was also shown by Cramer and Horning (84), Starkey and Schmidt (363) and Tolenaar (382) who obtained the effect in castrated male mice, young mature females, immature males and females, adult females, and adult ovariectomised females. It is uncertain whether or not this effect of androgens is exerted directly on the adrenals or is due to modification of pituitary activity.

The effect of oestrogen on the mouse adrenal appears to be more complex. Martin (275) used a presumably impure preparation (oestrin) and found that over short periods it did not affect the X-zone. This result is in keeping with the observed fact that the development of the X-zone is independent of, and not apparently influenced by, the presence of the ovaries. Martin also found, however, that prolonged treatment of immature castrated males and normal and spayed females, led to degeneration of the zone. This result is perhaps analogous to the gradual disappearance of the zone seen in unmated normal females. Martin ascribed the effect to the depression of the gonadotrophic function of the pituitary-body known to follow chronic oestrogenisation. His interpretation, however, presupposes that the gonadotrophic substance controls the development of the X-zone, whereas the contrary is indicated by Deanesly's (96) observation that the X-zone does not develop in the dwarf mouse in which, according to Smith and MacDowell (357) the gonadotrophic is the least deficient of the pituitary functions. By contrast, Martin also noted that prolonged injections of "oestrin" to normal immature males led to continued development of the X-zone. Burrows (42) also found that prolonged treatment of the normal male mouse with crystalline oestrogens led to the appearance of the

**X-zone** This effect may have been due to the occurrence of a castrate-like condition following testicular atrophy caused by depression of the gonadotrophic function of the pituitary, but this interpretation assumes that chronic oestrogenisation does not affect the adrenocorticotrophic activity of the pituitary body, or whatever hypophyseal factor is responsible for X zone development, or at least affects it less than the gonadotrophic activity. Cortical enlargement in mice oestrogenised for moderate periods has also been described by Deanealy (97). Results not dissimilar from those of Martin were obtained by Lacassagne and Raynaud (250) who found that oestrogenisation for short periods did not affect the X zone, but that prolonged treatment produced changes in the adrenals ascribable to depression of hypophyseal activity. Degenerative changes in the adrenals of chronically oestrogenised mice have also been described by Cramer and Horning (85) and Danner (90).

The fact that the X zone degenerates during the first pregnancy has led several workers to investigate the effects of corpus luteum hormone. Experiments were carried out by Martin with extracts of corpus luteum, which were found not to affect the condition of the X-zone. A similar result was also obtained for crystalline progesterone by Tolenaar (382) and Howard and Gengradom (211).

**Rat** The sex dimorphism in size of the adrenals of the rat has been referred to on p 205, the gland being larger in the female than in the male. We may now examine the extent to which this dimorphism is dependent on gonadal hormones. According to Korenchevsky, Dennison and Hall (239) testosterone does not have any effect on the size of the adrenals in normal male or female rats, but Hall (174) subsequently reported that "the effect of male hormones on females was to produce adrenals of similar size to those of normal un.injected males," thus causing the sex dimorphism to disappear. This conclusion agrees with that of McEwen, Selye and Collip (234) who found that injection of testosterone decreased the size of the adrenals in normal females by about one third, thus reducing them to the same size as the glands in the male, which themselves were unaffected by the treatment. In keeping with this result it has been shown that in castrated males, the hypertrophy of the adrenals which follows removal of the testes (see p 209) can be reduced or abolished by the administration of androgenic urine extract, androsterone, androstenediol, *trans*-dehydroandrosterone, testosterone, testosterone propionate, androstenedione and androstenediol (full references can be found in Hall and Korenchevsky 175). The histological appearance of the cortex is also restored to normal if moderate doses are used. The effects of androgenic substances in ovariectomised females are much less definite. According to Korenchevsky (234) injection of androgens often leads to some decrease in size, but this effect is less marked in the case of testosterone. As a general conclusion from these experiments it may be said that the sex dimorphism in the size of the adrenals of the rat is due largely to suppression of the male adrenal by testis activity, and that the effect can be reproduced in the gonadectomised male by appropriate treatment.

Comparatively little work has been done on the effect of oestrogens on the adrenals of rats. According to Selye, Collip and Thomson (348) enlargement

of the adrenals is only caused by oestrone when corpora lutea are present Selye, Collip and Thomson (348) and Ellison and Burch (120) agree in stating that administration of oestrogens fails to cause hypertrophy of the adrenals in hypophysectomised rats, a result which indicates clearly the involvement of the pituitary body in the response seen in intact rats Deanesly (97) noted that oestrogenisation of male rats leads, in the early stages, to cortical enlargement and, in the later stages, to atrophic changes consequent on the general disorganisation of pituitary function due to adenomatous changes According to Korenchevsky and Dennison (235, 237) administration of large doses of oestrogens causes adrenal enlargement in normal male rats but not in castrates It is likely that this effect is due to the coincident depression of testicular activity caused by the administration of oestrone, and the consequent appearance of castration changes in the adrenals Lauson, Heller, and Sevringhaus (255) found that administration of oestrogens for 19 days did not affect the size of the adrenals in ovariectomised rats Schilling and Laqueur (342) noted that injection of oestrone increased adrenal weight in thyro-hyperplastic female rats

*Squirrel* According to Zalesky, Wells, Overholser and Gomez (401) the administration of testosterone does not delay the atrophy of the adrenals which follows hypophysectomy in the ground squirrel

III THE EFFECTS OF THE ADRENAL GLANDS ON THE GONADS (a) *Effects of adrenalectomy* Complete removal of all adrenal cortical tissue leads to death of the animal within a short time Thus, pigeons (Gourfein, 164), fowl (Herrick and Torstvert, 188), and ducks (Parkes and Selye, 305, Bülbring, 33) survive only a few hours after the completion of the operation In the rat, it was originally stated by many workers up to 1928 that a high proportion of animals survived indefinitely after adrenalectomy, but more recent work has confirmed the isolated earlier observations that adrenalectomy is almost always fatal in this species, the animals dying within a few weeks of complete removal of the adrenal cortical tissue Thus, Pencharz, Olmsted and Giragossintz (308) found that all of 62 rats died within 2 to 18 days of the operation Freed, Brownfield and Evans (139) and Kutz (248) made similar observations Gaunt (147) emphasised the variation in different colonies of rats In four different colonies, 95 per cent of the rats died within 3 weeks of double adrenalectomy, the average survival time being 7 days In a fifth colony, 50 per cent lived for 30 days or more, the average survival time being 14 days for those that died He observed no significant sex difference in survival time Firor and Grollman (130) found that the mean survival time of the rat increased up to 9 days with increasing size of the animal Schultzer (345) recorded a mean survival time in 77 rats of 5.7 days, with a range of 3-12 days The older records of prolonged survival in rats after adrenalectomy appear to have been complicated by incomplete operations and the use of strains possessing much accessory tissue Mice behave similarly (Firor and Grollman, 130) Most rabbits die within a short time of operation (11 days or less) but a few survive long periods probably owing to the presence of accessory tissue In guinea pigs the complete operation is extremely difficult For the cat, Elliot's (117) original observations, indicating a survival

period of 6 to 23 days, have been confirmed by many more recent workers, 7 to 11 days being the accepted average figure. Dogs survive about the same time, the average period shown by Rogoff and Stewart's (329) extensive observations being about 7 days.

In these circumstances observation of the effect of complete adrenal deficiency on the gonads and thence on the reproductive processes presents some difficulty, since observations must be made on completely adrenalectomised animals, in the short period before death, on animals suspected of having accessory tissue, or on animals kept alive on cortical extracts or corticosterone which may or may not affect the gonads.

*Fowl* According to Herrick and Tortsveit (188) adrenalectomised fowl kept alive on cortical extract and sodium chloride showed testicular atrophy so markedly that in the course of a few weeks they had the appearance of capons.

*Mouse and rat* Freed, Brownfield and Evans (139) adrenalectomised a series of male rats between 26 and 30 days old. At death a maximum of 6 days later, the testes were degenerating and smaller than those of the controls. In this type of experiment, however, it is difficult to distinguish specific effects on the gonads from changes due to the general failing condition of the animals. The observations of Lewis (258), Deanesly (94), Castillo (68) and Schiffer and Nice (341) on the length of the oestrous cycle and the breeding performance in adrenalectomised mice and rats were probably made on incompletely adrenalectomised animals, since the survival time was considerable. More convincing experiments were reported by Wyman (398) who adrenalectomised 41 mice and studied their oestrous history by the vaginal smear technique. Twenty three died of acute adrenal deficiency and 16 of these had complete cessation of vaginal cornification in the month between operation and death. Examination of the ovaries showed that ovulation had also been suppressed. Eight rats died of chronic adrenal insufficiency and 6 of these had prolongation of the dioestrous period. Ten rats lived until autopsied 3 to 4½ months after operation, and 8 of these showed no serious disturbance of the cycle. Seven of this group were shown to have gross accessory cortical tissue. In another experiment, 28 adrenalectomised rats were mated, 15 became pregnant and 13 had normal litters. Eleven of these had gross accessory cortical tissue. Corey and Britton (83) found that the oestrous cycle was completely inhibited in 19 out of 22 adrenalectomised rats, but that it reappeared in adrenalectomised rats maintained on cortical extract. Kroc and Martin (244) record that of 18 adrenalectomised rats, 6 showed no return of the oestrous cycle after the removal of the second adrenal, while 8 showed one and 4 showed two oestrous type vaginal smears. Martin (276) obtained substantially similar results, and also found that adrenalectomy caused cytological changes in the pituitary gland and decreased the content of gonadotrophic hormone. The author attributed the reproductive derangements to the pituitary changes. This conclusion is in keeping with Dessau's (101) observation that the ovaries of doubly adrenalectomised rats respond normally to the administration of gonadotrophic extracts. However, Kutz, McKeown and Selye (249) showed that adrenalectomised rats maintained

in good health by the administration of sodium chloride experienced normal oestrous cycles, and Martin and Fazekas (277) found that adrenalectomised rats similarly maintained had a normal pituitary histology and gonadotrophin content as well as normal reproductive cycles

The effect of adrenalectomy on pregnancy seems to follow the same principles Britton and Kline (29) reported that pregnant rats which have been adrenalectomised fail to go through the normal processes of parturition, abortion is common, and lactation does not follow Dessau (101) found that adrenalectomy of the rat in the first half of pregnancy terminates the pregnancy Normal litters might, however, be produced if the operation were delayed until the second half of pregnancy According to Tobin (381) normal gestation and parturition may proceed in the absence of the adrenals, the percentage of successes being proportional to the stage of pregnancy at which the operation is carried out McKeown and Spurrell (285) found that adrenalectomy of the rat in the first 9 days of pregnancy led to non-implantation or to reabsorption, a salt diet usually prevented this result since most adrenalectomised pregnant rats maintained on salt delivered normal young, which, however, they were unable to rear owing to the failure of lactation

It seems clear, therefore, that the effect of adrenalectomy on the ovary and on pregnancy, whether or not it is exerted through the pituitary body, is a result of loss of general condition rather than of the lack of a specific gonadergic substance produced by the adrenals Most probably, as suggested by Long and Zuckerman (266), the changes in the accessory organs, involving considerable changes in their water metabolism, are dependent on a normal salt and water balance in the animal generally

*Cat and rabbit* Immediate adrenalectomy is said to inhibit the ovulation which would otherwise take place in cats following injection of gonadotrophic substances (Friedgood and Foster, 143) or after mating (Friedgood, 142), but the latter author inclines to the view that the effect is due to the general disturbance caused by the operation, since adrenalectomy of rabbits immediately after mating does not inhibit the ovulation which takes place some ten hours later Adrenalectomy of the pregnant cat usually terminates pregnancy within 48 hours (Britton and Kline, 29)

(b) *Effects of adrenal extracts* The work cited in the previous section makes it evident that the administration of cortical extracts allows the ovary of the adrenalectomised rat to continue its normal functions, but that the effect is a general somatic one and not due to special action of cortical hormones on the ovary It remains now to examine the effects of adrenal extracts on the gonads and thence on the accessory organs of intact animals The direct effects of such extracts, and particularly of desoxycorticosterone, on the accessory reproductive organs, are dealt with on p 236 in dealing with gonad-like effects of the adrenals The work to be considered in this section has largely been inspired by the observed facts of the adreno-genital syndrome in man and has dealt mainly with the effect on the gonads of (a) the typical life-maintaining extracts and substances of the adrenal cortex, and (b) other extracts made by methods designed to obtain different types of substance, if present

*Survival prolonging extracts* The increasing availability of active (survival prolonging) cortical extracts naturally led to experiments on the possibility of influencing the gonads of intact animals. Several apparently positive results were obtained, but these, as Grollman (169) points out, must have been due to chance, individual variation or non-specific constituents of the extracts. Certainly, more recent and carefully controlled experiments have failed to demonstrate any effect of cortical extracts on age of maturity, length of oestrous cycle or fertility.

Cleghorn (74) injected young mice daily for four weeks with a cortical extract made by the method of Swingle and Pfiffner. There was no significant change in the weight of the testis, the age of opening of the vagina or the length of the oestrous cycle as compared with controls. Schirrmeister (343) on the other hand, reported that repeated subcutaneous injections of adrenal cortex extracts in infantile mice produced enlargement of the reproductive tract and histological changes in its epithelium.

Corey and Britton (81, 82) claimed to have produced precocious maturation of the gonads in young rats by injection of Swingle and Pfiffner's extract. Changes were more marked in the ovaries than in the testis. Later, these authors (83) reported that administration of cortical extracts to normal rats might stimulate, depress or have no effect on the oestrous cycle.

Several other workers claim to have obtained positive results, including Fitzhugh (133), who found that cortical extract produced hypertrophy of the ovaries of young female rats, he did not, however, find that early maturity was produced in immature male rats. Hall, Chamberlin and Muller (176) reported that the administration of adrenal cortex extract to mature rats just before mating increased the incidence of pregnancy and the number of foetuses. The main action was on the female. The body of evidence that cortical extracts as ordinarily prepared have no effect on the gonads, is, however, considerable. Connor (78) was unable to confirm Corey and Britton's early result. Gaunt and Parkins (152) treated young rats and fowl over long periods with daily doses of 5 to 20 dog units of survival prolonging cortical extracts without influencing the reproductive system. Howard and Grollman (212) also concluded that a moderate excess of the hormone of the adrenal cortex has no appreciable influence on the size of the gonads, the oestrous cycle or on pregnancy. They also remarked, having in mind the masculinising tumours of the adrenal in man, that no signs of intersexuality occurred in the treated mice. Atwell (10) was unable to stimulate the ovaries of hypophysectomised rats by the injection of cortical extracts.

Pottenger and Simonsen (315) purified the benzene soluble fraction discarded in the process of preparing cortin by the method of Swingle and Pfiffner. They obtained a white amorphous powder which caused, they claim, increased testis weight and spermatogenesis when injected into male rats. Good results, which the authors consider may or may not have been due to the presence of "cortin," were also claimed in clinical trials on cases of cryptorchidism and arrested development of the testes and external genitalia.

*Gonadotrophic extracts of the adrenal* There have been persistent reports



of experiments which suggest that some gonadotrophin-like substance is present in adrenal tissue. Thus, results suggestive of those obtained with gonadotrophic pituitary extract have been recorded by several workers who have used alkaline or similar essentially aqueous extracts of adrenal tissue. Casida and Hellbaum (66) found that pyridine extracts of the adrenals of mares and geldings caused ovarian stimulation in immature test rats. Allen and Bourne (2) made an aqueous extract of kangaroo adrenals and obtained luteinisation of the ovaries of immature rats. Similar results have been obtained by Hoffmann (198, 199) who described the active substance from the adrenals of cattle as having chemical properties very similar to those of pituitary gonadotrophins, and Kliatschko (223) who worked with extracts of pig, cattle and human adrenals, the last of these being much the most active. These authors emphasize that the gonadotrophic substance is distinct, biologically and chemically, from the recognised cortical hormones. Unfortunately, they have worked with rather minimal biological responses and whether or not the presence of small amounts of gonadotrophic substance in the adrenals is substantiated it may be taken as certain that the dramatic effects of aqueous pituitary extracts on the gonads are not simulated by similar adrenal extracts.

*Miscellaneous experiments* Eaton, Insko, Thompson and Chidester (114) described an increase in weight of the testes of young chicks fed with desiccated adrenal cortex, while Chidester, Eaton and Thompson (72) conclude that similar treatment expedites sexual maturity in young female fowl. These conclusions receive no support from other knowledge as to the general nature of the active substances of the adrenal cortex. Klein (222) made a saline extract of adrenal cortex which he claimed stimulated the sexual organs of male rats and depressed those of female rats.

IV ADRENAL-LIKE ACTIVITY OF THE GONADS The first evidence that the gonads might, under certain conditions, show some adrenal-like activity was derived from the observation that, in dogs, the survival time after adrenalectomy was influenced by the sexual condition of the animal at the time of operation. More recently, the availability of large amounts of gonadal hormones in crystalline form has allowed the discovery that certain of these substances, notably progesterone, have corticosterone-like properties.

(a) *Effects of sexual condition on survival after adrenalectomy* Age and sex Sisson and March (353) found that female rats survived adrenalectomy slightly longer than males. The difference was slight, but was consistent at all age groups examined. They found that age, however, was a much more important factor than sex. Thus, at 20 days old, male rats survived an average of 5.0 days, females 6.4 days, at 70 days old, males survived 15.25 days and females 19.33 days. Gonadectomy experiments make it unlikely that the influence of age is connected with gonadal puberty changes. Kroc (243) also found that females survived slightly longer. He suggests that this could be associated with the larger size of the female adrenal, but it would seem equally reasonable to maintain that its larger size indicates a greater requirement on the part of the female and possibly, therefore, a shorter survival period after complete deprivation.

*Gonadectomy* The effect of gonadectomy on survival after adrenalectomy has been examined by several workers. Carrying out castration simultaneously with adrenalectomy Carr (64) found a slightly longer survival period than after adrenalectomy alone, but it is doubtful if the slight difference had any significance. Mendez (286) carried out castration a few days before removing the adrenals and again obtained an insignificant increase in survival time. Since the size changes in the adrenals which follow gonadectomy in rats take some weeks to appear, experiments on previously castrated animals are of more interest than those in which the gonads and adrenals are removed simultaneously. Gonadectomy of male or female rats 6-20 weeks before adrenalectomy was found by Kroc (242, 243) to increase the survival period slightly (2-3 days) but insignificantly. Differences in weight loss were also small. In rabbits, Handovsky and Tamman (179) and Mira and Fontes (289) found that previous castration increased resistance to adrenalectomy. Kroc (243) notes that castrated male rats survive about as long as normal females, and suggests that this may be due to the fact that the adrenals hypertrophy in castrated males to a size approximating that seen in the normal female.

*Anostrus* A most interesting correlation between the condition of the gonads and the effects of adrenalectomy has been shown by Bülbring (34) who used drakes for the assay of cortical extracts. The drake is remarkable in showing a regular and very short survival period after the removal of the adrenals (Parkes and Selye, 305, Bülbring, 33), and Bülbring found that the amount of cortin required to keep the birds alive for a 16 hour period after operation varied greatly at different times of year. She was able to correlate the amount with the pronounced changes which take place in the size of the testes during the summer, the amount of cortin required being proportional to the size of the testes and decreasing by about five-sixths when the sudden drop in testis weight occurs during May and June. These results might be interpreted by supposing that testosterone is antagonistic to the action of corticosterone, but an alternative explanation is possible. The decrease in size of the drake testes after the breeding season is accompanied by atrophy of the seminiferous tubules, and by a change rather than a cessation of the endocrine activity. Male hormone effects disappear at the end of the breeding season, but, on the other hand, the plumage undergoes changes which are somewhat similar to those produced by oestrogens and which do not appear in the castrate, so that the small testes of the post-breeding season period must be supposed to elaborate substances different from those produced in the breeding season. It might be inferred, therefore, that the substance produced during the eclipse period can supplement corticosterone, rather than that the testosterone produced during the breeding season is antagonistic. Experiments on castrated drakes would, of course, settle this question.

A case of seasonal sensitivity to adrenalectomy in mammals was described by Britton (28) who showed that there is a marked difference in the survival of *Ardomys monax*, the North American marmot, according to the time of year at which double adrenalectomy is carried out. Hibernating marmots did not suffer

immediate ill-effects from adrenalectomy, but at the usual time of awakening in April such animals showed signs of severe adrenal insufficiency and soon died. The critical period clearly corresponds with the time of renewed activity in the gonads, but it is uncertain how far it is dependent thereon.

*Oestrus* As recorded below the occurrence of oestrus at or about the time of operation in the dog, which ovulates and becomes pseudopregnant spontaneously, promotes survival after adrenalectomy. In the ferret, on the other hand, which does not ovulate spontaneously and remains in oestrus indefinitely in the absence of mating or other ovulation-producing stimulus, Gaunt and Hays (148) found that the occurrence of spontaneous or induced oestrus accelerated the appearance of symptoms of adrenal insufficiency.

*Pregnancy and pseudopregnancy* Carr (64) found that the fact of a rat being pregnant did not increase its period of survival after adrenalectomy. Firor and Grollman (130), on the other hand, adrenalectomised ten pregnant rats and found an average survival time of 15 days, twice the usual period. These authors note that those rats which gave birth after the operation failed to come into lactation.

As long ago as 1912 Stewart (367) found that pregnant cats survived adrenalectomy longer than non-pregnant cats. Corey (79, 80) and Rogoff and Stewart (332), however, failed to confirm this finding, and Corey also found that lactation did not prolong survival time in cats. Collings (77), however, obtained very definite positive results in cats made pregnant or pseudopregnant following artificially induced ovulation. The average survival time following removal of the second adrenal was 23 days, nearly three times the average for non-pregnant animals. The discrepancies in these results on the cat are probably due to the fact that the cats were operated on at different stages of pregnancy. In the cat the corpora lutea begin to undergo involution about a month after the beginning of pseudopregnancy (Liche, 259) and considerably before the end of normal pregnancy. Since Corey used cats in various stages of pregnancy while Collings used them soon after mating, the mean prospective life of the corpora lutea at operation was probably very different in the two sets of experiments.

Rogoff and Stewart (330) found that pregnancy had a marked influence in prolonging the survival of dogs after adrenalectomy. Of 17 pregnant animals, 12 survived much longer than the average for males and non-pregnant females, the average being 22 days as compared with 7 days for the controls. These authors suggested, on histological grounds, that these results might be attributed to the presence of the corpus luteum during pregnancy. Later, Rogoff and Stewart (331) recorded that the occurrence of "heat" at or about the time of operation prolonged survival almost as well as pregnancy, survival periods of 36, 32, 19 and 64 days being recorded. They did not apparently recognise that heat in the unmated bitch ushers in a period of pseudopregnancy, which in this species lasts as long as true pregnancy and is associated with the same degree of development of the corpora lutea. This observation as to the effect of pseudopregnancy was confirmed by Pfiffner, Swingle and Vars (311) and Swingle, Parkins, Taylor and Morrell (370), and a similar state of affairs has been found



in other organs, during the phases of reproductive activity. These changes are undoubtedly conditioned by the gonadal hormones, notably by oestrogens and they are reminiscent in a general way of those caused by corticoid substances, but are essentially dissimilar in that they depend largely on tissue specificity in response, or are paralleled in a small degree only by similar changes in other tissues of the body.

During the follicular phase of the normal cycle in the rhesus monkey there is an increase in the water content of the uterine endometrium (van Dyke, 386). In the pig-tailed macaque the development of the sexual skin into a large oedematous swelling during the follicular phase, and its sudden regression at the time of ovulation, causes changes in water metabolism sufficiently pronounced to be clearly reflected in the body weight and in the amounts of urine excreted (Krohn and Zuckerman, 245). The same retention of water presumably occurs in other monkeys, such as the baboon, in which there is a similar swelling of the sexual skin in the follicular phase, and to a lesser extent in rhesus monkeys where the sexual skin undergoes slight swelling, corrugation, etc. Analogous processes presumably take place in the comb and wattles of cocks when they enlarge in response to androgenic stimulation.

In women, a gain in body weight often precedes menstruation (Sweeney, 369, Okey and Stewart, 300). Less frequently, there is a generalised or local oedema preceding or accompanying menstruation (Thomas, 376, Atkinson and Ivy, 9). It is clear that such changes might be due to one or more of several factors, and their relation to the phenomena seen in lower primates is uncertain. However, Peterson and Milles (309) found that menstruation in normal women was accompanied by changes in the permeability of the skin capillaries, which may well be the mechanism by which the sexual skin oedema in monkeys is brought about. Moreover, Thorn, Nelson and Thorn (380), who studied the sodium chloride and water balance in normal women, found that there was a retention during the intermenstrual and pre-menstrual phases, and an increased excretion at the onset of menstruation.

(c) *Corticoid effects of gonadal hormones* The lethal effects of adrenalectomy seem to depend primarily on disturbance of the salt and water metabolism, and Thorn, Engel and Eisenberg (378) have shown that the salt-retaining activity of various cortical substances is related in a general way to their effectiveness in promoting survival after adrenalectomy. The adrenals, however, also play an important rôle in the regulation of carbohydrate metabolism and work-capacity. These various activities of the adrenals are dissociable, as shown by the fact that the three best known of the many active substances isolated from the adrenal cortex have a different effectiveness in different directions. Thus, corticosterone and 11-dehydro-17-hydroxycorticosterone (substance E of Kendall) are very effective in influencing carbohydrate metabolism (Long, Katzin and Fry, 265, Ingle, 213) and work-capacity (Ingle, 214), but have little effect or even an adverse effect on the retention of sodium and water, and, therefore, on survival after adrenalectomy (Ingle and Thorn, 215), desoxycorticosterone has little effect on carbohydrate metabolism (Long, Katzin and Fry,

265) or in maintaining work-capacity (Ingle, 214) but is most active in retaining salt and water, and, therefore, in promoting survival (Thorn, Engel and Eisenberg, 379) The possible corticoid effects of the gonadal steroids must, therefore, be considered in the light of these dissociable functions of the cortex.

**Oestrogens** Oestrogens are generally agreed to have no favourable effect on the survival of adrenalectomised animals Martin (276), Swingle, Parkins, Taylor, Hays and Morrell (371), and Emery and Schwabe (123) obtained negative results with small doses of oestrogenic extract, as did Wells and Greene (393) with oestradiol, diethylstilboestrol, triphenyl ethylene and 4,4'-dihydroxy-diphenyl According to Kroc (242), small doses of oestrone to non-ovariectomised females increased survival time (from 14.3 days to about 19 days) in one series of rats, but in another the survival time was inversely proportional to the dosage Injection before as well as after adrenalectomy prolonged survival Injection of oestrone to castrated and non-castrated adrenalectomised males decreased survival time Injected females showed vaginal cornification, so that the presence of cortical hormones is not essential for this response

According to Cavanaugh and Gaunt (70) and Pfeiffer and Hooker (310) oestrogenic preparations are not merely not beneficial but are actually toxic Thorn and Engle (377) found that oestrone in substantial dosage had no effect on the survival of adrenalectomised dogs, though 17 mgm. of oestradiol caused retention of sodium and chloride in a female patient with Addison's disease and both oestrone and oestradiol had this effect in normal dogs In an experiment of a rather different kind, Cramer and Horning (86) found that whereas administration of oestrogen after adrenalectomy decreased survival time, chronic oestrogenisation before the operation increased it

Injection of oestrogens into ovariectomised monkeys leads to retention of water in the sexual skin swelling similar to that seen in the follicular phase of the normal cycle (Zuckerman, 407) This response is strictly specific in nature, since the administration of cortical extract, testosterone or progesterone, does not maintain the sexual skin swelling after the administration of oestrogen is discontinued According to Astwood (8) the injection of oestrogen to the spayed rat causes a transient rise in the water content of the uterus, which reaches a peak at 6 hours after injection and is back to normal in 12 hours A similar result for the uterus was found by Zuckerman, Palmer and Bourne (411) who also showed that there were similar water-content changes in the skin, and reciprocal ones in muscle, heart, brain, pancreas and gut, the whole experiment being a conclusive demonstration of shift of water in the body caused by oestrogen administration

**Androgens** Testosterone is non toxic and non beneficial to adrenalectomised ferrets (Gaunt and Hays, 148) The same applies to androstenedione and dehydroandrosterone in rats (Wells and Greene, 393) Thorn and Engle (377) found that testosterone propionate had no beneficial effect in adrenalectomised dogs, though it caused retention of sodium in normal dogs Androstenedione and androstenediol in the doses used (40 mgm) were without effect even in normal dogs Spurr and Kochakian (361) found that urinary androgenic extract,

androstenedione, testosterone acetate and testosterone propionate decreased the survival time of adrenalectomised rats, the effect being proportional to their androgenic activity

*Progesterone and allied substances* The evidence that the presence of functional corpora lutea ameliorates the effects of adrenalectomy, and the similarity in chemical structure between progesterone and the cortical substances, suggested that progesterone might give positive results in the ordinary tests for corticoid activity. Early investigators (Swingle, Parkins, Taylor, Hays and Morrell, 371, Cavanaugh and Gaunt, 70, Schacher, Browne and Selye, 339, D'Amour and Funk, 89, Steiger and Reichstein, 364) of this possibility obtained negative results, probably owing to the use of inadequate dosage. More recently a long series of reports of positive results makes it certain that the administration of large doses of progesterone promotes the survival of adrenalectomised animals. Two milligrams per day of progesterone maintained adrenalectomised ferrets in good health (Gaunt and Hays, 148, 150). It was less effective in adrenalectomised rats, 1 to 2 mgm per day being required to extend life up to 20 days in half the animals (Gaunt, Nelson and Loomis, 151), and it did not modify their response to excess water. Greene, Wells and Ivy (168), however, found that a high proportion of adrenalectomised rats would survive on a daily dose of 4 mgm progesterone. Pregnanediol and allopregnanediol have no corticoid-like activity (Wells and Greene, 393) in the Everse-de Fremery work-capacity test. Waterman, Danby, Gaarenstroom, Spanhoff and Uyldert (391) found that progesterone gave negative results in rats (4 mgm daily) and in dogs (up to 12 mgm daily). Ingle (214) also found that progesterone did not maintain the work-capacity of adrenalectomised rats. Thorn and Engel (377) report that 20 mgm of progesterone to normal male dogs resulted in a decrease of sodium and chloride excretion about equal to that given by 0.8 mgm desoxycorticosterone or 4 mgm corticosterone, and according to Cantarow and Rakoff (61) progesterone has an effect similar to that of desoxycorticosterone in expediting the secretion of sodium and chloride into the peritoneum of normal dogs. In adrenalectomised rats, Schwabe and Emery (346) found that 1 mgm daily of progesterone would maintain life, Bourne (24) that 0.5 mgm daily of progesterone doubled the survival time, and Emery and Greco (122) that progesterone had the same order of activity in maintaining life as desoxycorticosterone acetate.

Work has also been carried out with compounds closely related to progesterone. Thus, Steiger and Reichstein (365) observed that 21-oxy-progesterone, in which a  $\text{CH}_2\text{OH}$  group is substituted for the terminal methyl group in progesterone, has definite corticosterone-like activity, about one-third as great as that of the substance itself.

V GONAD-LIKE ACTIVITY OF THE ADRENALS (a) *Gonad-like effects of the adrenals in experimental animals* The clinical evidence that adrenal tissue can, under certain conditions, produce substances capable of inducing the development of rudimentary male organs and secondary sexual characters in the human female has prompted efforts to produce similar effects in experimental

animals This work has mainly taken the form of attempts to demonstrate the secretion of androgens or oestrogens by the adrenals of male or female rats or mice Owing to the absence of secondary sexual characters in ordinary laboratory animals, work has been concentrated on the accessory organs This has made progress difficult in the male, since the prostate and seminal vesicles of rats and mice respond only to comparatively large doses of androgens

*Androgenic action in the male* A starting point for the work was provided by Price's observation (316) that whereas in the adult male rat castration leads to immediate degeneration of the prostate, especially of the epithelium, castration of the newborn rat is followed for some weeks by considerable growth and differentiation of the gland In keeping with this observation Price (319) later showed that young ventral prostate tissue grafted into young castrated males undergoes development till the host reaches an age of about 40 days Howard (204, 205) correlated the continued development of the prostate gland in the castrated newborn rat with cellular enlargement and other changes in the inner part of the adrenal cortex, and suggested that the adrenals of the young rat exerted an andromimetic function

Burrill and Greene (37) in further analyses of this possibility, carried out experiments on young rats by *a*, castration, *b*, castration and adrenalectomy, and *c*, adrenalectomy Adrenalectomy alone did not affect the early development of the prostate so that androgenic action by the adrenals does not seem to be essential in the presence of the testes, but removal of the adrenals in addition to the testes prevented the early differentiation seen after castration alone, and the authors concluded that andromimetic activity was exerted by the adrenals in the absence of the testes Gersh and Grollman (155) carried out a similar experiment and arrived at contrary results They were unable to find any difference between the prostate glands of castrated and castrated-adrenalectomised animals Burrill and Greene (39) repeated their experiments on a larger scale and confirmed their previous results Further, they claimed (41) to show that the adrenal androgen differs from the testicular one in not being destroyed by the liver Howard (208, 209) added further evidence concerning this question She found that adrenalectomy at three weeks old of castrated rats caused a 50 per cent decrease in weight of the prostate as well as degenerative histological changes These changes in the prostate were not prevented when the rats were maintained at a normal growth rate by the administration of desoxycorticosterone acetate She concluded that the adrenals secrete a physiologically appreciable amount of androgen at this stage of development It may be noted that Burrill and Greene, and also Howard, maintained the adrenalectomised rats by sodium chloride therapy, while Gersh and Grollman administered adrenal extract, but it is unlikely that this difference can account for the discrepancy

On the balance of the evidence it may be concluded provisionally that the adrenals of the immature rat are able to exert androgenic activity, though they do not apparently play an essential part in this respect if the testes are present In the adult rat, however, it seems to be agreed that castration reveals no alternative source of androgenic substance It has not been conclusively shown,



however, that this early differentiation of the rat prostate is dependent upon androgenic stimulation. Possibly, as Grollman (169) suggests, the rudimentary gland possesses some inherent growth potentiality.

The seminal vesicles of the rat behave differently from the prostate. According to Price (316) there is some slight enlargement after early castration, but both this author and Howard (207) agree that there is no differentiation. If the development of the prostate of the newborn castrated rat is really due to androgen of extra-gonadal origin, possibly the seminal vesicles have a higher threshold requirement.

If the adrenal glands of the normal young rat have androgenic activity it might be expected that glands caused to hypertrophy by the administration of hypophyseal adrenotrophic hormone would show the same activity more strongly. Actually, accounts of work on these lines preceded the papers by Burrill and Greene, and Gersh and Grollman. Davidson and Moon (93) first reported that the administration of adrenotrophic extracts to young castrated rats caused development of the prostate as seen by size increase and histological stimulation, and to a lesser extent of the seminal vesicles. The adrenals were much hypertrophied, and the authors attributed the changes in the accessory reproductive organs to the enhanced androgenic activity of the hypertrophied adrenals. In a later paper Davidson (92) showed that the adrenotrophic extract had no effect on the prostate if the animal was adrenalectomised but that the response was not abolished by hypophysectomy. These experiments, confirmed by Nelson (295), correlate well with the later ones of Burrill and Greene.

Less work has been done on mice, but according to Howard (204) there is a marked difference between the rat and mouse in the response to adult castration. In the rat, as mentioned above, degeneration of the prostatic and vesicular epithelium follows very rapidly after adult castration. In the mouse, on the other hand, although there is a considerable size decrease in both glands much of the epithelium of both glands retains its high columnar character and a fairly normal general character for as long as forty days after adult castration. The author ascribes this difference to the presence in the castrated mouse of the X-zone which is assumed to have greater andromimetic power than ordinary cortical tissue in the adult, and which mimics the testis and delays the post-castration changes in the accessory organs. In a more detailed paper, Howard (207) came to the following conclusions: "The reactions of the seminal vesicles of mice to castration vary according to the age at operation, and are related to the histological condition of the adrenal cortex. In mice castrated at 5 days of age, when the seminal vesicles are relatively undifferentiated, these structures undergo a marked degree of differentiation which includes the formation of columnar epithelium. This reaction is preceded by hypertrophy of the adrenal X-zone. In mice castrated at 21 days of age, when the columnar vesicle epithelium has differentiated, the X-zone hypertrophies and the condition of the vesicles undergoes little alteration for a considerable period, which may extend for as long as 100 days. In mice castrated after sexual maturity, when the primary X-zone has disappeared, the vesicles undergo a degeneration which remains incomplete

during the period in which the secondary X zone differentiates in the adrenal cortex. One hundred days after castration the secondary X zone has frequently disappeared from the cortex, and in these cases the vesicles are in a state of marked and uniform degeneration." Howard (206) later showed that adrenalectomy of young castrated mice prevented the differentiation of the vesicular epithelium which is not abolished by castration. A most interesting experiment bearing on this subject has been reported by Spiegel (360). He castrated three male guinea pigs a few days after birth. Several years later the rudimentary penis started to grow and two ultimately effected copulation, with the formation of vaginal plugs. At autopsy the reproductive tract and accessory glands were found to be similar to those of the normal adult male, and in each case there was a large adenoma of the adrenal cortex. This experiment seems to afford a clear case of androgen secretion in physiologically effective amounts by the hyperplastic adrenal.

*Gynaecogenic action in the male* There is as yet little evidence suggestive of oestrogen secretion by the adrenal of the normal or castrated male mouse or rat. Oestrogen induced changes in the male reproductive tract proper require large dosage and the males of these particular species are poorly equipped with female rudiments.

*Androgenic action in the female* In considering the source of any androgens demonstrated to be present in the female, as for instance by Price's (317, 319) observations that male prostate tissue grafted into normal adult female rats remains in a functional state for several months, it must be remembered that the ovary has been shown to be capable of producing androgens. Thus, Lipschütz (260), Hill (192) and Deanealy (95) reported that under certain conditions ovarian grafts caused development of the accessory glands of castrated males, and Parkes (304) found slight androgenic activity in crude extracts of ovary. In spite of this complication there is now good experimental evidence that the adrenal of the female may show androgenic activity. The female rat is especially suitable for investigation of the endogenous production of androgens because it possesses a well marked male rudiment, a female prostate. These rudiments which were first described by Marx (278, 279) respond to androgenic stimulation in the same way as the male gland and are capable of development to a condition in which they are histologically identical with the ventral lobe in the male (Korenchevsky, 232, Korenchevsky and Dennison, 238, Korenchevsky, 233). The gland is not affected by oestrogens. Korenchevsky's observations were confirmed by Hamilton and Wolfe (178), Deanealy and Parkes (99) and Witschi, Mahoney and Riley (395).

Price (318) studied the development of this prostate gland in the female and found that, as in young castrated males, it undergoes growth and differentiation for a period of about 40 days, when regression begins. She pointed out that this period is similar to that during which the juvenile cortex of the rat adrenal retains its distinctive character in both males and females, and suggested that the early development of the female prostate is due to androgenic activity on the part of the juvenile cortex. Burrill and Greene (38) transplanted im

mature male prostatic tissue into normal and ovariectomised immature females and concluded that the female prostatic tissue has a lower threshold requirement of androgenic stimulation than the male prostate, and that there is an extra-ovarian source of androgen in the female. Later the same authors (40) showed that neither ovariectomy nor adrenalectomy alone stopped the initial development of the female prostate, but that removal of both organs simultaneously did so. They concluded that both the ovary and the adrenal of the immature female rat are capable of producing physiologically demonstrable amounts of androgen.

Somewhat analogous observations have been made on the female preputial glands, which are only slightly affected by gonadectomy or injection of oestrogens, but which hypertrophy readily on the injection of androgens (Korenchevsky, 234). Noble and Collip (297) found that hypophysectomy caused atrophy and injection of pituitary extracts hypertrophy of the glands. The latter effect was almost abolished by the removal of adrenals and ovaries, and appeared to be mediated through the adrenals or ovaries, or both, according to the type of extract. It is likely, therefore, that under the influence of stimulation by pituitary extracts both adrenals and ovaries can produce enough androgen to cause growth of the preputial glands.

In this connection attention may be called to a highly suggestive series of experiments which are not usually considered in relation to the adrenal-gonad relationship. It is well known that under certain circumstances masculine stigmata, mainly hypertrophy of the clitoris and appearance of the preputial horns, appear in female guinea pigs. Stemach and Kun (366) produced this result in association with extensive luteinisation of the ovary following x-r-radiation or administration of pituitary extracts. The result was confirmed by Guyenot, Ponce, Dottrens, Vallette and Trollhet (171) and Guyenot, Ponce and Wietrzykowska (173) using pituitary extracts, and by Guyenot, Ponce and Trollhet (172), Greene and Burrill (165) and Bradbury and Gaensbauer (27) using urine of pregnancy extracts. Greene and Burrill (166) also obtained evidence of stimulation in prostate tissue grafted into female rats injected with urine of pregnancy extracts. In view of the work of Lipschütz (260), Hill (192) and Deanesly (95) who showed that ovarian grafts into castrated males often caused marked development of the atrophic male accessory organs and that the effectiveness of the graft in this connection was proportional to the degree of theca luteinisation undergone by the graft, it was first supposed that the masculinising effect of the luteinised ovary in the female guinea pig was due wholly to the production of androgens by the lutein tissue. Guyenot and his collaborators, however, showed that the masculinising effect could be obtained in the ovariectomised animal by injecting pituitary extracts, but not by injecting urine of pregnancy extracts. The latter result was confirmed by Greene and Burrill (166) in their female rats carrying male prostate grafts. They conclude, therefore, that while the effect of the urine of pregnancy extracts is entirely mediated through the ovaries that of the anterior pituitary extracts depends partly or wholly on some other mechanism. Since the urine of preg-

nancy extracts used contained no other active principle than chorionic gonadotrophin, while the anterior pituitary extracts may well have contained adrenocorticotrophin, it may be surmised that the effect of the latter in ovariectomised animals was due to stimulation of the adrenals, leading to the secretion of physiologically effective amounts of androgen. The growth effect of oestrogens on the clitoris (Papanicolaou and Falk, 302, Turner and Burkhardt, 385), like that of the urine of pregnancy extracts, seems to be dependent on ovarian changes.

*Gynaecogenic action in the female* The fact that in the usual course of events the ovariectomised animal does not show symptoms of oestrus even by the most delicate criteria known, complete vaginal cornification of the rat or mouse, precludes the possibility that the adrenals can have any considerable oestrogenic effect after removal of the ovaries. Nevertheless, phenomena highly suggestive of weak oestrogenic activity have been observed. Thus, it is well known that the time of opening of the vagina in the pubertal rat is only slightly affected by ovariectomy. According to Wade and Haselwood (388) removal of the adrenals alone has no more effect than removal of the ovaries, but removal of the adrenals as well as the ovaries may postpone opening for as much as two months. It seems, therefore, that ovaries and adrenals are alternative sources of the stimulus causing vaginal opening. This conclusion is similar to that arrived at by Burrill and Greene in relation to the early development of the female prostate in the rat. Other suggestive observations have been made about the behaviour of the reproductive tract after ovariectomy. Thus, Kostitch and Telebakovitch (240) found that even after complete ovariectomy there was a subdued rhythm in the vaginal epithelium of the mouse, a preponderance of mucus, leucocytes, or nucleated epithelial cells following each other in regular order, Frank, Goldberger and Salmon (138) and Geist and Salmon (153) noted that in some women after ovariectomy the vaginal contents and epithelium do not show the regressive changes usually associated with deprivation of oestrogen. There are also peculiarities in the response of ovariectomised animals to minimal doses of oestrogen. Tuisk (384) reported that in ovariectomised mice injected daily with small amounts of oestrogen the otherwise continuous vaginal cornification was occasionally, but irregularly, interrupted by invasions of leucocytes. A similar, but more precise observation was made by del Castillo and Calatroni (69) who found that vaginal cornification occurred periodically in ovariectomised rats injected with a constant low dose of oestrogen. Zuckerman (405) and Bourne and Zuckerman (25) confirmed these observations, and showed conclusively that whereas constant heavy dosage of oestrogen to the spayed rat led to persistent cornification, daily administration of a threshold dose led to the occurrence of cornification at intervals similar in length to the dioestrous period of the normal female. Di Paola (301) obtained analogous results with a daily dose of 0.1  $\mu\text{g}$  of oestradiol benzoate. The threshold dose varied in different rats, being between 0.5  $\mu\text{g}$ . and 1.5  $\mu\text{g}$  of oestrone. Zuckerman (406, 408) also showed that removal of the pituitary body did not abolish such artificial threshold cycles, but that in adrenalectomised rats maintained on desoxy cortico-

sterone, the phenomenon could be reproduced only with the greatest difficulty. Zuckerman (404) observed a similar state of affairs in ovariectomised rhesus monkeys, in most of which the daily injection of 10  $\mu$ g of oestrone led to periodic bleeding such as can be produced by oestrogen withdrawal. This bleeding occurred at intervals of similar length to the normal intermenstrual period of the rhesus monkey, and indicated the occurrence of a periodic physiological drop in the oestrogenic stimulation given by a constant daily dose, caused either by a periodic decrease in sensitivity to oestrogen or a periodic withdrawal of an endogenous source. Recurrent menstruation-like bleeding has also been reported by Reynolds, Kaminester and Schloss (324) in an ovariectomised woman maintained on regular dosage of oestrogen. In continuation of the monkey experiments, Bourne and Zuckerman (26) have described morphological and histological changes in the adrenal of the rat associated with oestrus in the normal animal and with artificial oestrus in the ovariectomised rat injected with a threshold dose of oestrone. They concluded that the adrenal glands in the rat fluctuate in size in a cycle corresponding to the oestrous cycle, and that, though the changes are independent of both the pituitary body and the gonads, they exercise a direct effect on the accessory reproductive organs. It is not at present certain whether this cyclic response of the accessory reproductive organs to exogenous oestrogenic stimulation is due to the periodic production by the adrenal of small amounts of oestrogen, or, as suggested by Long and Zuckerman (266), periodic fluctuation in the adrenal controlled water metabolism causing fluctuations in the sensitivity of the accessory organs to oestrogenic stimulation.

In addition to the above observations, there is some evidence that under special conditions the adrenals may exert considerable oestrogenic activity in the ovariectomised animal. In this connection, the observations of Woolley, Fekete and Little (397) are of extreme interest. It is generally agreed that removal of the ovaries in the young animal largely prevents the appearance of mammary cancer even in the most susceptible strains. Woolley and his co-workers, however, found that if mice of the aba strain were ovariectomised at birth, more than 25 per cent developed mammary cancer at an average age of 522 days, as against about 50 per cent in untreated females. This result suggested strongly that considerable amounts of oestrogen ultimately appeared in the mice ovariectomised at a very early age, and this deduction was confirmed by the fact that the uterus, thin and anaemic six months after ovariectomy, was hypertrophied and hyperaemic in animals killed more than one year after operation, at which time several showed not merely vaginal cornification but cyclic appearance thereof. Woolley and his co-workers made careful examinations for regenerated ovarian tissue with negative results, but the adrenals were found to be enlarged and nodular, the degree of the hypertrophy increasing with increasing age. The experiments, taken in conjunction with the somewhat similar ones of Spiegel on male guinea pigs (see p 227) undoubtedly suggest strongly that very early gonadectomy may result ultimately in highly effective androgenic or oestrogenic activity on the part of the adrenal.

Further progress could probably be made along these lines by the injection

of corticotrophic extracts into ovariectomised animals. A beginning in this direction was made by Moon (290) who obtained vaginal changes which he considered to indicate oestrogenic stimulation in immature ovariectomised rats injected with adrenocorticotrophic preparations. Further information was obtained by Nelson (295) who was able, by the injection of adrenocorticotrophic hormone, to cause enlargement of the uterus, vagina and mammary glands in gonadectomised or hypophysectomised, but not in adrenalectomised rats.

(b) *The adreno-genital syndrome* It is not intended here to enter into a full discussion of the adreno-genital syndrome of which full reviews have been given from time to time by Broster and Vines (32), Grollman (169), Broster and his collaborators (31), Kepler (219), Rowntree and Ball (335) and Simpson (350). These together with the historical account given by Rolleston (333) should be consulted for details. The present brief account is intended solely to orientate the clinical observations on the adreno-genital syndrome as it occurs in man to the larger problem of the relationship between the adrenal cortex and the gonads in higher vertebrates.

The evidence discussed above suggests that in experimental animals the adrenals are capable, at least under certain circumstances, of producing oestrogens or androgens in physiologically effective amounts. According to present knowledge, however, spontaneous dysfunction of the adrenals on a scale adequate to cause disturbance of the reproductive organs and secondary sexual characters is seen only in man. The resulting condition, the adreno-genital syndrome, is of great interest, and in spite of the simplicity of the relevant biological conceptions, of considerable complexity. It is clear that the excessive production by the adrenals of gynaecogens in females, or of androgens in males, will lead to precocious or hypersexuality. The excessive production of the heterosexual hormones, on the other hand, will tend to cause inversion of the sexual organs and characters. The excessive production of both will lead to a complicated combination of precocity and inversion.

It is a general rule that the degree to which inversion of the reproductive organs can be produced depends on their degree of differentiation at the time when the inverting stimulus becomes operative. Thus, the effectiveness of any heterosexual adrenal activity depends on the age at which it begins, the earlier the more effective. It may be premised, therefore, that the clinical results of adrenal hyperactivity depends first on whether androgens or oestrogens or both are produced in excess, and whether the derangement appears prenatally, prepubertally, or in the adult.

*The rôle of the foetal adrenal* In keeping with the above principles it has been suggested that prenatal abnormality of the adrenal cortex accounts for certain types of congenital hermaphroditism and that such types are the extreme expression of the adrenogenital syndrome. According to Broster *et al.* (31) "the common type of pseudohermaphroditism is a sex inversion of the female due to a cortical androgenic hyperfunction occurring before the end of the fifth month of foetal life". These authors consider that right up to the time of puberty sexual development is controlled by the adrenals, rather than by the gonads,



In boys showing the typical adreno-genital syndrome there is premature appearance of masculine hair, enlargement of the penis and external genitalia, and deepening of the voice. Grollman (169) states that the testis does not undergo development and does not show spermatogenesis, but Lissner (261) has reported to the contrary. The changes are generally similar to those described by Kunstader (246) as having been produced in two boys by the administration of androgen (testosterone). In both sexes there is unusual muscular development, but this is more marked in the male, in which according to Harris and Plewes (183) it occurs in 60 per cent of cases. The fact that there is usually lack of testicular development shows that even in males the condition is not one of genuine precocious puberty and is distinguished thereby from the syndrome of *pubertas praecox* arising from tumours of the pineal. However, the extent to which androgenic stimulation may directly or indirectly affect spermatogenesis is not certain, and a condition closely resembling full precocious puberty cannot altogether be excluded as a theoretically possible consequence of adrenal hyperfunction.

The essential association between the adrenal tumour or hyperplasia and the abnormal bodily changes is demonstrated by the fact that, in what is now quite a large number of cases in boys and girls, successful removal of the offending adrenal tissue has resulted in regression of the abnormal bodily changes. Striking examples of this effect have been recorded by Collett (76), Glynn and Hewetson (159), Walters, Wilders and Kepler (389), Lissner (261) and Lukens and Palmer (268). Recurrence of the tumour, or the further growth of metastases, results in a recrudescence of the bodily changes. Reilly, Lissner and Hinman (323) collected records of thirteen successful operations for the removal of the neoplastic or hyperplastic adrenal in cases of the syndrome in pre-adolescent girls.

*Virilism in adolescents and women.* In adults, masculinisation of women due to hyperplastic or neoplastic changes in the adrenals is a well defined syndrome, and full descriptions have been given by Glynn (158), Gallais (146) who gave the syndrome its present name, Glynn and Hewetson (159), Gordon Holmes (201), Fordyce (134) and Broster and Vines (32). It is evident from the reports cited by Kepler (219) and from the work of Goldzieher and Koster (160) and Broster and Vines (32) that the condition can occur as the result of simple adrenal hyperplasia as well as of neoplasia, and its essential connection with the adrenals rather than with the ovary is well shown by Kovacs' (241) report of the development of virilism after ovariectomy. The following description of the changes in adult women is given by Grollman (169): "The adreno-genital syndrome as it occurs in women is marked by an appearance of secondary male characters and a retrogression of the female sex characters. Among the former are (1) hypertrichiasis of the male type, (2) changes in bodily contour, (3) changes in the larynx, (4) hypertrophy of the clitoris, and (5) the assumption of the psychological outlook of the male. The retrogression of female characteristics is marked by a cessation of menstruation and the loss of other female characteristics which are replaced by the masculine characters already noted." These changes are



generally similar to those described by several authors as following the therapeutic administration of androgens to adult women. The ovaries of a woman from whom a large cortical malignant adenoma was removed were described by Rosset (334) as containing many primordial and Graafian follicles, but no corpora lutea. After successful operation there is re-feminisation with loss of hair, development of the breasts and return of menstrual function. According to Broster and Vines (32) and Goldzieher and Koster (160) the adrenal hyperfunction can be relieved by the removal of one gland, even when no tumour is present.

*Adreno-genital syndrome in men* The adreno-genital syndrome rarely occurs in adult men, but the few reported cases are of great interest in demonstrating the ambisexual potentialities of the adrenals. From the fact that abnormal masculinisation is usually the chief symptom in boys, girls and women it might be supposed that adrenal hyperfunction in men would lead to super-masculinisation. Such a result, however, seems to be rare, though Macera (269) has reported such a case. The usual findings appear to be the reverse. Bittorf (20) and Holl (200) recorded feminism, testicular atrophy and gynaecomastia in men with cortical neoplasms. Holl's subject was of special interest in being the father of two sons, and in recovering from the gynaecomastia and other symptoms after the removal of the tumour. Holl also reported gynaecomastia in a boy with a very large adrenal tumour. Bittorf's case was further described by Mathias (282). Zum Busch (412) and Weber (392) reported a case of gynaecomastia and actual lactation in a man with a large "hypernephroma" and another similar one was recorded by Lisser (262). Simpson and Joll (352) have also reported the presence of a highly excessive amount of oestrogen in the urine of a man showing atrophy of the genitalia, impotence and gynaecomastia in association with a malignant adrenal tumour. The tumour was removed, causing a decrease in excretion of oestrogen, but later, with the growth of metastases which led to the patient's death, the amounts of oestrogen in the urine rose to as much as 6400 m.u. per litre. Biochemical observations on this case of considerable interest were reported by Burrows, Cook, Roe and Warren (43) (see p. 245). Two instances of testicular atrophy and gynaecomastia not associated with adrenal tumour, but thought to be due to adrenal hyperplasia, were described by Glass and Bergman (157). Neither of these patients excreted excessive amounts of oestrogen, but the androgen/oestrogen ratio was low for males. A case has also been described by Broster (30) in which feminism as defined by feminine body contours, small external genitalia and sparse pubic hair, was relieved by unilateral adrenalectomy after which the voice became deeper and the genitalia larger, and there was growth of secondary sexual hair.

In some of these cases the changes described as constituting feminisation, including female disposition of fat, do not seem to have been different from those which are seen in eunuchoidism, and which might have been expected to follow the atrophy of the testes. On the other hand, the latter symptom, and especially the gynaecomastia, must be considered highly significant. These changes, however, cannot be considered as occurring invariably in men with adrenal tumours (Crooke and Callow, 87).

*Résumé of adreno-genital syndrome* If we accept the idea that the adrenals can secrete both androgens and oestrogens from the time they appear in the foetus right up into adult life, that these substances can affect the reproductive organs, and that hyperplastic or neoplastic changes in the adrenals may result in a greatly enhanced secretion of one or the other, then the theoretical possibilities emerge which are listed in table 1. Of these twelve types, the four (5, 7, 10, 11) in italics are well recognised and have been described above. Of the others, the hyper isosexual adults (9, 12) would presumably not be very distinct types, the isosexual precocious girl (8) would be as described by Bennett (18), types 1 and 4 would be extreme cases of isosexuality, and the remaining types (2, 3, 6) would include various kinds of "hermaphrodite". According to Broster *et al.*,

TABLE 1

TIME OF OPERATION OF ADRENAL HYPERFUNCTION	GENETIC SEX	NATURE OF ADRENAL HYPERFUNCTION	
		Primarily androgenic	Primarily oestrogenic
Foetal	Male	1	2 'Hermaphrodite Femaleness superimposed on basically male organs
	Female	3 'Hermaphrodite Maleness superimposed on basically female organs	
Prepubertal	Male	5 <i>Precociously masculinised boy</i> ? Complete precocious puberty	6 Feminised boy
	Female	7 <i>Masculinised girl especially as regards secondary sexual characters</i>	8 Precociously pubertal girl Isosexual precocity
Adult	Male	9 <i>Hyper-masculinised man</i>	10 <i>Feminised man</i>
	Female	11 <i>Woman with virilism</i>	12 Hyper-feminised woman

2 and 6 are very rare owing to the lesser oestrogenic activity of the male adrenal and the greater stability of the male reproductive system.

*Nature of the adrenal tissue undergoing hypertrophy* There has been much discussion of the precise nature of the adrenal tissue which undergoes hyperplastic or neoplastic changes, resulting in disturbance of the sex characters. The idea that some special tissue is involved is supported by the fact that neither hyperplasia nor neoplasia of the cortex necessarily leads to aberration of the sex characters, and that where such aberration occurs there is not usually any evidence that an excess of cortical hormones proper is being produced. Grollman (169) in this connection has called attention to the inner cortical layer which develops in the human foetus and undergoes involution after birth. This zone is probably represented in the adult by the thin layer of cells surrounding the medulla which

are readily distinguished from the cortex proper (Goormaghtigh, 161) Grollman (169) homologises this zone, under the name androgenic tissue, with the X-zone of the mouse. He considers that it is the seat of the masculinising tumours found in the human subject, and that malignancy of the cortex proper does not lead to any aberration of the sexual characters. Grollman's views, however, are not strengthened by his later conclusion (Gersh and Grollman, 155) that neither the "juvenile cells" of the rat, nor the X-zone of the mouse perform any androgenic function. In contrast to the idea that a special zone of tissue is involved, Broster and Vines (32) have put forward the view that there is present in the foetal adrenal of both sexes a special fuchsinophil substance which disappears at birth and appears post-natally in easily detectable amounts only when the adrenal undergoes hyperplasia or neoplasia of the type which leads to masculinisation.

In some instances, the offending tissue may apparently be outside the adrenal proper, as in the woman described by Saphir and Parker (337) who showed a typical clinical condition of adrenal virilism associated with apparently normal adrenals and aberrant adrenal tissue in the ovaries.

*Relation with pituitary and other syndromes* It is outside the scope of the present review to consider the relation of the adreno-genital syndrome to pubertas praecox, Cushing's syndrome of basophilism, the Archard-Thiers syndrome, and the like. This subject has been dealt with in detail by a number of other writers, including Kepler (219), Grollman (169), Lescher (257), Simpson (350) Broster *et al* (31) and Crooke and Callow (87). It must, however, be pointed out, that when aberrations of other endocrine organs, notably of the pituitary gland, are involved either primarily or secondarily, along with those of the adrenals, the effects on the reproductive organs and secondary sexual characters will be accompanied by a great variety of other bodily changes — obesity, plethora, etc — which may render the subject totally abnormal quite apart from sexual peculiarities. For analogy it may be recalled that gonadal and thence changes in the other reproductive organs are only one of the many symptoms making up the syndrome of Simmonds' disease. It may also be remarked that the disturbance of the carbohydrate metabolism seen in the Archard-Thiers syndrome (diabetes of bearded women) is suggestive of over-production of the ordinary cortical hormones as well as of androgens by the neoplastic or hyperplastic adrenal.

The summary in table 2 is taken from Reilly, Lissner and Hunman (323).

(c) *Effects of cortical extracts and hormones on the accessory reproductive organs and secondary sexual characters* *Cortical extracts* Even before it was demonstrated that the adrenals might affect the reproductive organs of gonadectomised animals various kinds of adrenal extracts had been examined for activity similar to that of gonadal hormones. Thus, Müller (292) reported that he had been able to cause hypertrophy of the uterus of normal and thymectomised rats by the injection of protein-free cortical extracts. Asher and Klein (7) recorded similar results in females and also obtained hypertrophy of the reproductive organs in male rats. Klein (222) claimed that adrenal extracts accelerated the develop-

ment of the reproductive organs in male rats but inhibited it in female rats. Loewe, Marx, Rothschild and Voss (204) reported that an adrenal extract, not effective in cases of Addison's disease, caused hypertrophy of the rudimentary prostate in female rats. Observations have also been made in cases of Addison's disease receiving treatment with cortical extracts. Thus, Edwards, Shimkin and Shaver (116) noted swelling and tenderness of the breasts in a man after three months' treatment. There was recession of the glands after the end of treatment. According to Carr (65) and Hartman, Lockwood and Brownell (184) life-prolonging extracts of adrenals do not maintain lactation in adrenalectomised rats. When the characteristic cortical hormones were isolated, prepared in

TABLE 2  
*Relation of adreno-genital syndromes with other conditions*

	TUMORS OF THE ADRENAL CORTEX	OTHER CONDITIONS CAUSING SIMILAR SYNDROMES
In intra-uterine life	Pseudohermaphroditismus	
In infancy		
Boy	Sexual precocity	Pineal tumor Testicular tumor Cerebral tumor
Girl	Pseudo-sexual precocity with pubic hair Enlarged clitoris	True sexual precocity Adult genitalia Early menstruation Granulosa-cell tumor of the ovary
In adult life		
Male	Gynecomastia Femininization	Eunuchism
Female	Virilism or hirsutism Masculinization	Cushing's disease Oat-cell tumor of thymus Arrhenoblastoma of ovary

pure form, and their close chemical relation to the gonad hormones shown, immediate investigation was made as to whether they possessed similar biological properties. Only desoxycorticosterone has so far been available in amounts allowing of extensive tests.

*Androgenic activity of desoxycorticosterone* Hooker and Collins (202) reported that desoxycorticosterone, in a daily dose of 20 mgm, caused a significant growth of the comb of the capon, and, in a daily dose of 0.5 mgm, stimulated the seminal vesicles and prostate gland of castrated mice, and the seminal vesicles but not the prostate of castrated rats. Hooker and Collins' mammalian experiments were not very convincing, and the results were contrary to those of Greene and Burrill (167) who made a careful histological study of the effects of desoxycorticosterone on the ventral prostate of castrated and castrated-ad

renalectomised rats, and came to the conclusion that the substance had no demonstrable androgenic activity by this test, the most sensitive mammalian one known. Negative results on rats and chicks were also obtained by Paschkis (306). Chamorro (71) on the other hand found that administration of desoxycorticosterone delayed the atrophy of the accessory reproductive organs of the male mouse after hypophysectomy. Less direct evidence of androgenic activity has been brought forward by Selye (347) who showed that desoxycorticosterone causes regression of the X-zone of the mouse adrenal, and by Burdick and Konanz (36) who reported that the action of desoxycorticosterone on the uterus and blastocysts in early pregnancy is similar to that of testosterone. According to Dantchakoff (91) administration of desoxycorticosterone acetate to the embryonic or newborn rat does not affect sexual development. When combined with testosterone, however, it produces precocious development of the mammary gland and augments the effects of the androgen on the genitalia of the female. On the basis of present information it is extremely unlikely that desoxycorticosterone is a sufficiently good androgen to account for the observed androgenic activity of the adrenal. Probably the same applies to corticosterone, since the addition of the 11-hydroxy group is not likely to promote androgenic activity.

*Oestrogenic activity of desoxycorticosterone* According to Salmon (336) administration of desoxycorticosterone in doses of 40 mgm to 60 mgm to post-menopausal women produces changes in the vaginal epithelium similar to those produced by oestrogens. Speert (359) also obtained results which suggest that desoxycorticosterone is oestrogenic in rhesus monkeys to the extent of increasing vaginal desquamation, causing sexual coloration and stimulating lobule-alveolar growth in the mammary gland. He found that bleeding of the oestrogen-withdrawal type followed cessation of injection.

*Progestational activity of desoxycorticosterone* Desoxycorticosterone is chemically more similar to progesterone than to any of the other gonadal hormones, differing from it only in having a  $\text{CH}_2\text{OH}$  group in place of the  $\text{CH}_3$  in position 21. This similarity is paralleled by the fact that progesterone has certain corticoid properties (see p 224) and that desoxycorticosterone has been shown to evoke response in the typical tests for progestational activity. Miescher, Fischer and Tschopp (287) found that 10 mgm of the substance evoked a typical response in the uterus of the sensitized immature rabbit (Clauberg test for progesterone). Similarly, de Fremery and Spanhoff (141) found, by the same technique, that 15 mgm of desoxycorticosterone evoked about the same response as 2 to 3 mgm of progesterone. Heuverswyn, Collins, Williams and Gardner (190) confirmed these results and added the interesting information that the adrenal substance, like progesterone, is able to potentiate the mating response to oestrogens in the spayed guinea pig, 10 mgm being about equal in effect to 1 mgm of progesterone. Torstveit and Mellish (383) obtained a similar potentiation in the guinea pig with both desoxycorticosterone and cortical extracts. Robson (328), in a more extensive study, showed that desoxycorticosterone also resembled progesterone in maintaining pregnancy in spayed mice and in spayed and hypophysectomised rabbits, in modifying the response of the rabbit's uterus to pituitrin, and in

suppressing the oestrous cycle in mice Zuckerman (409), investigating the question in primates, found that large doses, 15 to 40 mgm daily of desoxy corticosterone, like progesterone, inhibited oestrogen withdrawal bleeding in the monkey. In only one of three monkeys, however, was there a typical progestational proliferation of the uterine endometrium, 1120 mgm had been administered to this animal.

(d) *Presence of gonadal hormones and analogous substances in the adrenals*  
The first impressive indication that the adrenal contained appreciable amounts of substances with an action similar to that of the gonadal hormones was provided by Engelhart (125, 126, 127, 128) who showed that crude lipid extracts possessed both oestrogenic and progestational activity in tests on ovariectomised rats and immature rabbits. These observations were confirmed by Callow and Parkes (60) who further showed that extracts from horse adrenals were the most active and that the two activities could be fractionated by the method introduced by Allen and Meyer (3) for separating oestrogen and progesterone in extracts of corpus luteum. These authors found that 200 grams of fresh horse adrenal contained progestational activity equivalent to that of 1 mgm of progesterone. They also showed that both activities were present in fractions of adrenal extracts discarded in the course of the industrial manufacture of cortin and it therefore seemed unlikely that the oestrogenic and progestational activity present in the adrenals had any essential connection with the characteristic cortical hormones. Further observations were made by Fischer and Engel (131) who reported that the adrenals contained 33 units of oestrogen and 5 units of progestational activity per kilogram. This work was brought to an early and highly satisfactory conclusion by Beall, who isolated both progesterone and oestrone in pure form from extracts of adrenal. The isolation of progesterone and incidentally of allopregnanolone, was carried out almost simultaneously by Beall and by Reichstein, and was the subject of a joint note (Beall and Reichstein, 17). In a further paper Beall (13) gave details of the chemical steps and identification, and showed that progesterone probably accounted for the whole of the progestational activity of the original extract. Later Beall (14, 16) reported the isolation of oestrone from each of two batches of adrenal material discarded in the manufacture of cortin. The hormone was separated from the phenolic ketonic fraction as its benzoate or dinitrobenzoate. The phenolic non ketonic fraction was also oestrogenic, so that the whole of the oestrogenic activity of adrenal extracts is not due to the presence of oestrone. It is likely, however, that the active substance of the phenolic non ketonic fraction is oestradiol or oestriol or both, as in the case of extracts of horse testis (Beall, 15).

As regards the androgenic activity of the adrenals, the situation is less clear cut. Crude extracts of  $\alpha$  adrenal were found by Parkes (304) to have a slight androgenic activity as tested on the capon comb by the sensitive unctio n technique, but Carnes (63) failed to obtain a similar result with acetone-ether extracts of human foetal adrenal. Hodler (196, 197) claims to have produced stimulation of the prostate and seminal vesicles of castrated guinea pigs by the administration of alkali extracts of  $\alpha$  adrenals, the action being similar to that

of androgens So far, however, no highly active androgen has been isolated from adrenal extracts Reichstein (322) isolated a weak androgen, adrenosterone, which by virtue of a 15-keto group stands rather apart from the regular series of androgens It is uncertain whether this substance can account for the whole of the observed androgenic activity of adrenal extracts

It is impossible to say at the present stage whether the gonad-like activity of the adrenals in the gonadectomised animal is due to the secretion into the circulation of oestrone, progesterone and adrenosterone It may, however, be noted that evidence obtained from the experimental animal suggests that appreciable androgenic activity may be exerted, while evidence of progesterone-like activity is missing In adrenal extracts, by contrast, progesterone is relatively abundant, while androgens are weak and scarce

(e) *Excretion of androgens and oestrogens after gonadectomy and in adrenal dysfunction* It is well known that in man and certain other mammals, the urine of both sexes contains appreciable amounts of androgenic and oestrogenic activity It was originally believed, without any real evidence, that the substances responsible for these activities originated in the gonads It is now clear, however, that at least part of the substances must be of extra-gonadal, and is probably of adrenal origin

*Androgens and oestrogens of animal's urine* Little is as yet known about the excretion of androgens and oestrogens by laboratory animals Smelser (355) and Schmidt (344) observed that detectable amounts of oestrogen were present in the urine of oestrous guinea pigs, and Emmens (private communication) finds that young female guinea pigs excrete about 0.2 I U per day in the urine, males excrete a similar amount According to Emmens, male rats excrete much less than guinea pigs, about 0.005 I U per day Koch (226) reported that rat urine contains less than one I U of androgen per litre The source of the oestrogens in rat and guinea pig urine does not seem to have been ascertained, but the lack of striking sex difference suggests that the gonads are not the primary source

A somewhat similar condition is found in monkeys According to Allen, Diddle, Burford and Alder (1) chimpanzees excrete 49 to 142 mouse units of oestrogen in 24 hours, there being some cyclical variation during the menstrual cycle Dorfman and van Wagenen (112) found up to 2.5 I U of oestrogen and up to 4.7 I U of androgen per 24 hours in the urine of male rhesus monkeys These authors also found both substances in female monkeys Fish, Young and Dorfman (132) obtained up to 13.5 I U per 23 hours of androgen and up to 135 I U of oestrogen from the urine of male chimpanzees Figures obtained for oestrogens by these workers were similar to, though rather higher than, those given by Allen *et al* Small amounts of androgens were found during the follicular phase of the cycle in females, but not during the luteal phase, though the authors do not attach significance to this on the data available In general, they found that male chimpanzees excreted about twice as much androgen as females and somewhat less oestrogen Highly significant experiments have been reported by Dorfman and van Wagenen (113) These authors found a great

increase in urinary oestrogens and a considerable increase in urinary androgens during pregnancy in the rhesus monkey. After removal of the foetus and ovaries no significant change in oestrogen or androgen excretion occurred. With the subsequent expulsion of the placenta, oestrogen excretion dropped to the non pregnancy level or slightly below, while androgen excretion showed little change for about a month. From these most interesting experiments it may be concluded that, in the monkey, the greater part of the oestrogens excreted during pregnancy are of placental origin, but that the androgens, like the residual oestrogens, are derived neither from the placenta nor the ovaries and are probably of adrenal origin.

More is known about the urine of farm animals. According to Koch (226) bull and ram urine contains less than one IU and 4 IU of androgen per 24 hours. In large-scale chemical studies Marker (270) obtained androsterone, dehydroandrosterone and oestrone from bulls' urine. He also (271) found the same three substances in steers' urine, in about the same amounts. According to Nibler (296) non pregnant cows excrete small amounts of oestrogen, but data are not available on androgens, or for ovariectomised cows. Zondek's (403) remarkable observations showed that the stallion excretes large quantities of oestrogen, afterwards found to be largely or exclusively oestrone. By contrast, the urine of the gelding has little oestrogenic activity. It seems, therefore, that the androgens and oestrogens of bulls' urine are not of gonadal origin and are probably derived from adrenal secretions, while the oestrogen of stallions' urine seems definitely to be of testicular origin.

*Androgens of human urine* It is unnecessary here to deal with the literature on this subject in detail, the review by Callow (58) may be consulted for references, etc. It will suffice to say that there is but little differentiation in the amounts of activity present in the urine of men and non-pregnant women, as first shown for androgens by Womack and Koch (396), and the active substances present appear to be the same. Thus, Dingemanse, Borchardt and Laqueur (103) found 40 to 50 units of androgen per litre of men's urine and 30 to 60 units per litre of women's urine. Callow (57) found 29 and 26 units respectively, and Gallagher, Peterson, Dorfman, Kenyon and Koch (145) 63 to 68 units and 42 to 56 units. Similar observations have been made by other workers. The work of Butenandt and Dannenbaum (44), Butenandt, Dannenbaum, Hanisch and Kudezus (45), and Butenandt and Tscherning (46) showed that the androgenic activity of male urine was mainly or wholly accounted for by the presence of androsterone, dehydroandrosterone and androsterone. After a detailed investigation, Callow and Callow (51, 52, 53) reported that androsterone and dehydroandrosterone occurred in the urine of normal women in amounts similar to those found in men's urine. The actual amounts were—androsterone men 1.6 mgm per litre, women 1.3 mgm per litre, *trans*-dehydroandrosterone men 0.2 mgm per litre, women 0.2 mgm per litre. They also obtained the inactive but related aetiocholanolone in similar amounts from the two sources. *Iso*-androsterone was found in female urine by Pearlman (307). According to Nathanson, Towne and Aub (294) both boys and girls excrete similar small



amounts of 17-ketosteroids in early childhood, the amount increases with age, but more rapidly in boys

This lack of quantitative or qualitative sex differentiation in the excretion of androgens throws grave suspicion on the idea that they originate in the gonads, and the suspicion is strengthened by the fact that hypo- or hypergonadal activity does not seem to have any great effect on the amount excreted. In the extreme case of gonadectomy substantial amounts of androgen are found in the urine. The literature is reviewed by Callow, Callow and Emmens (54). Initial failures to obtain androgens from the urine of eunuchs and ovariectomised women were probably due to deficiency of technique. Positive results were, however, reported by Koch (224, 225), Kenyon, Gallagher, Peterson, Dorfman and Koch (218), Hansen (181, 182), McCullagh (283) and Hamblen, Ross, Cuyler, Baptist and Ashley (177). The highest value obtained by any of these workers was 22 I U per litre for eunuchs, and 61 I U per litre for ovariectomised women. Callow, Callow and Emmens (54) found androgens without exception in the urine of a series of 11 eunuchs and 18 ovariectomised women. They found a maximum androgenic activity of 28 I U per litre for eunuchs, and 59 I U per litre for ovariectomised women, and they concluded that the average amounts were below those for normal men and women, but that individual figures came within the normal range. From the urine of a eunuch Callow and Callow (53) obtained androsterone 0.5 mgm per litre, aetiocholanolone 0.9 mgm per litre, and *transdehydroandrosterone* 2 mgm per litre. The first two were thus present in smaller amounts than in normal men (see above), the third in considerably greater amount. Hirschmann (194) obtained the same three substances from the urine of ovariectomised women in amounts but little less than those present in the urine of normal women.

These results make it extremely unlikely that the androgens and related substances of the urine of normal men and women are exclusively of gonadal origin. It remains now to consider the evidence that they are of adrenal origin. The evidence, as in the case of the gonads, is derived from cases of hyper- and hypofunction. Callow, Callow and Emmens (54) found that in the case of Addison's disease, as with gonadal deficiency, the androgen excretion was on the whole low, but that many values were within the normal range. The authors point out, however, that the patients were receiving cortical extract, which may well have increased the amount of androgen excreted. The substances present have not been identified beyond the fact that they are probably 17-ketosteroids. Much more dramatic results have been obtained in cases of adrenal hyperplasia. Simpson (349) first reported that the urine of a case of virilism contained an excess of androgenic substance, and suggested that it might be of adrenal origin. Later, Simpson, de Fremery and Macbeth (351) recorded the results of the examination of the urines from a series of cases of virilism and pseudohermaphroditism. They found up to 400 I U of androgen per litre, and gave definite form to the idea that in certain circumstances the adrenals might produce physiologically effective amounts of androgens. Callow (56) reported the isolation of 110 mgm per litre of *transdehydroandrosterone* from the urine of a girl

of six with a large adrenal tumour. In a more detailed report on the case Crooke and Callow (87) recorded a maximum excretion of 850 mgm per litre of 17 ketosteroids, of which *transdehydroandrosterone* was the chief constituent. In the case of a man with an adrenal tumour Crooke and Callow record a 17 ketosteroid value of 64 mgm per litre. After removal of the tumour the excretion of 17 ketosteroid and androgen fell to normal. Other reports of excessive excretion of androgens in cases of adrenal tumour have been made by Cahill, Loeb, Kurzrok, Stout and Smith (49) 480 I U per litre, Kenyon, Gallagher, Peterson, Dorfman and Koch (218) 480 I U per day, and Slott (354) 2200 I U per litre. Lukens and Palmer (268) reported 160 I U per day in the urine of a masculinised adolescent girl, the androgen content fell to a fraction of this amount following the successful removal of the tumour. Masculinisation is found where these large amounts are present in women. Moreover, virilism in women presumed to be of adrenal origin, but not associated with malignancy, is often associated with the excretion of abnormal amounts of androgens, as shown by Kenyon, Gallagher, Peterson, Dorfman and Koch (218), Glass and Bergmann (157), Broster, Allen, Vines, Patterson, Greenwood, Marrian and Butler (31), Dingemans and Laqueur (104), and Callow (58). Koch (227) and Glass and Bergmann (157) have called attention to changes in the androgen-oestrogen ratio, caused either by increase of androgen or decrease of oestrogen. Three steroid substances were isolated from the urine of women with adrenal hyperplasia by Butler and Marrian (47, 48), namely, the weak androgen *isoandrosterone*, and the inactive compounds, *aetiocholanolone* and *pregnantriol*, the second of which is more closely related to the cortical hormones than to the androgens or *aetiocholanolone*.

In relating these results to the adrenal-genital relationship it must be remembered that, even if androgens and similar substances found in the urine of normal and gonadectomised men and women are wholly of adrenal origin, there is no proof that they are secreted by the adrenal as such. They may well be excretion products of the cortical hormones proper: testosterone, for instance, is almost certainly excreted partly as androsterone and *aetiocholanolone* (Callow, 50), and there is nothing inherently improbable in the idea that the gonadal and cortical hormones have common excretion products. It is unlikely, however, that the great amount of *transdehydroandrosterone* excreted in tumour cases can all be derived from cortical hormones, but there is no evidence as to the form in which the excess *transdehydroandrosterone* leaves the adrenal. It may be significant that Slot (354) failed to find androgenic activity in the tissue of the tumour of the case excreting 2200 I U per litre. However, it seems clear that the excreted androgen ascribable to adrenal activity is produced by the adrenal or in other tissues or fluids from adrenal products, and the end result, so far as androgenic activity on the part of the adrenals is concerned, is therefore the same.

*Oestrogens of human urine.* Knowledge concerning the distribution, quantity and nature of excreted oestrogens is analogous with that for the androgens, but is much less complete owing to the greater difficulties of extraction and characteri-

zation, and the smallness of the amounts present. As with the androgens, there is little sex differentiation in the amounts of oestrogen present in human urine and it usually occurs in the urine of menopausal women whose ovaries are atrophic (Kurzkrook, 247). The unexpected presence of oestrogens in male urine was observed more than 15 years ago by Laqueur, Dingemanse, Hart and de Jongh (251), but accurate comparison of the amounts present in the urine of men and women was only made possible by systematic work on the conditions necessary for the extraction of maximal amounts. (See Callow, Callow, Emmens and Stroud (55) for discussion.) Even now it is difficult to compare results obtained in different laboratories, and the only comparisons for male and female urines which will be mentioned here are those where the figures have been obtained simultaneously by the same author. According to Gallagher, Peterson, Dorfman, Kenyon and Koch (145) the average daily excretion of oestrogens of normal men amounts to 90–120 I U, and of women to 180–360 I U, there being in the latter some cyclic variation during the menstrual cycle. Borchardt, Dingemanse and Laqueur (22) found that by the best method of extraction the oestrogen content of the urine was 330 I U per litre for women and 160 I U per litre for men. Oesting and Webster (299), working on children, obtained up to 60 I U per litre for boys and up to 80 I U per litre for girls. Dorfman, Greulich and Solomon (111) found up to 95 I U per day and much less for girls. Callow, Callow, Emmens and Stroud (55) examined two batches of urine from each sex by the same technique and obtained values of 57 I U per litre and 86 I U per litre for the women's and 19 I U per litre and 27 I U per litre for the men's. According to Nathanson, Towne and Aub (294) both boys and girls of 3 to 7 years of age excrete small amounts of oestrogen. Subsequently, increased amounts appear, but the amount increases more rapidly in girls.

The nature of the oestrogen in women's urine is not definitely known, but according to Smith, Smith and Pincus (356) fractionation of the urine concentrate by the method used by Cohen and Marrian (75) for separating oestrone and oestriol, yields two fractions both of which were active, and it may be supposed therefore that both oestrone and oestriol are present in normal women's urine. Marker, Rohrmann, Wittle and Lawson (272) have isolated two isomeric hexahydro-oestradiols, which they consider to be reduction products of oestrone, from the urine of non-pregnant women. More is known about the oestrogens of male urine. Dorfman, Gallagher and Koch (110) on the basis of biological tests concluded that the oestrogens of human male urine included oestrone, and also another substance which was not oestriol. Confirmation of this view was provided by the notable work of Dingemanse, Laqueur and Mühlbock (105) who obtained 6 mgm of crystalline oestrone from 17,000 litres of men's urine. They report, however, that only one-third to one-half of the total activity passes into the ketonic fraction, so that an oestrogen other than oestrone must be present.

The source of the oestrogen of normal urine is uncertain, but as with the androgens, gonadectomy does not cause its disappearance. Thus, Frank, Goldberger and Salmon (138) note the presence of almost normal amounts of

oestrogen in the urine of each of twelve ovariectomised women Laroch, Simonnet and Huet (252) found varying amounts, and Eng (124) and Kemp and Pedersen Bjergaard (217) small amounts of oestrogens in the urine of ovariectomised women. Callow, Callow and Emmens (54) obtained oestrogenic activity from the urine of every one of 16 ovariectomised women at periods from one month to 27 years after operation. In some cases the amount was very small, and in all except one it was below normal. The exceptional case showed the high figure of 100 I U per litre. The occurrence of oestrogens in the urine of eunuchs is also established. Thus, Bingel (19) observed larger amounts than are usual in normal men, Eng (124) and Hansen (180) found small amounts. Kenyon *et al* (218) record 30 I U per day and 45 I U per day of oestrogen in the urine of two eunuchs. Similar amounts, 60 I U, were found by Dingemanse, Borchardt and Laqueur (103). Callow, Callow and Emmens (54) obtained oestrogen from the urine of each of 11 eunuchs, though the amounts present were very small. These observations make it extremely unlikely that the excreted oestrogens are wholly of gonadal origin even when the gonads are present, though one cannot entirely dismiss the possibility that their presence after gonadectomy is due to compensatory hyperactivity of some other organ.

There is now some evidence of the participation of the adrenals in oestrogen excretion, derived from studying the effects of hypo- and hyper-activity. Frank (136, 137) reported the presence of large amounts of oestrogen in the urine of women with adrenal cortical carcinoma. The amounts 1000–10,000 mouse units per litre were far above those seen in normal women or in cases of adrenal adenoma and hyperplasia. Saphir and Parker (337) found 5000 mouse units of oestrogen in the urine of a woman with adrenal carcinoma. Similarly, Burrows, Cook, Roe and Warren (43) obtained large amounts of oestrogen from the urine of a male patient with a malignant tumour of the adrenal. The substance was not isolated, but the chemical and biological results were concordant with its being oestrone. As a contrast to these observations on hyperactivity of the adrenals, Callow, Callow and Emmens (54) noted that oestrogen excretion was low in cases of Addison's disease.

In other work, the androgen/oestrogen ratio has been considered rather than the gross amounts of each. Thus, Glass and Bergmann (157) observed that in 10 women showing signs of virilism the ratio of androgen to oestrogen was increased, while in two men with gynaecomastia it was decreased.

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## PHLORHIZIN GLUCOSURIA

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Phlorhizin is a bitter glucoside,  $C_{21}H_{21}O_{10} + 2H_2O$ , derived from the root bark of apple, cherry, plum and pear trees. It is also designated phloridzin, phlorizin, phlorrhizin. The name is derived from the Greek—*phloios*, bark, *rhiza*, root. It occurs as minute white, or slightly pinkish crystals, of a silky texture, or as a pale, yellow, light crystalline powder, odorless, and having a bitter, but later a sweet, taste. It is sparingly soluble in cold, but freely soluble in hot water, from which it crystallizes on cooling. It is soluble in alcohol (1 part to 4) and sparingly soluble in ether. The solutions are levo-rotatory. At  $100^{\circ}C$ , it loses water, and at about  $107^{\circ}C$ , it melts. When heated to about  $130^{\circ}C$ , it becomes solid again and melts again at  $170^{\circ}C$ . At about  $200^{\circ}C$  it assumes a red color, due to the formation of rufin. Boiled with dilute acids, it is converted into sugar (phlorose) and phloretin. Exposed to air in the presence of ammonia, it assumes a purple color. Cold concentrated sulfuric acid dissolves it to a yellow solution and at  $25^{\circ}$  to  $50^{\circ}C$  the solution becomes red (108).

Phlorhizin was discovered in 1835 by the Belgian chemist, L. de Koninck, who used the material in the treatment of malaria, on the grounds that it was a bitter like other remedies which were effective in this disease. In 1839, Stas discovered that the material was a glucoside and could be broken down by acid hydrolysis into phloretin—a white crystalline compound,  $C_{11}H_{14}O_6$ , which is said to have febrifuge properties—and phlorose, subsequently proven to be identical with glucose.

Von Mering, in 1886, determined the important physiological property of phlorhizin—the production of a glucosuria when introduced into the animal body—and this initial discovery laid the groundwork for many worthwhile investigations.

The literature has been reviewed from time to time (21, 49, 80, 90, 91, 110), but no complete review in English has been forthcoming.

**Dosage.** Von Mering (97, 98) at first gave it to dogs in a mixture with ground meat. One gram of phlorhizin per kilogram caused 10 grams per cent of glucose to appear in the urine. He was also able to elicit a glucosuria in phosphorous-poisoned animals, and even in animals with the liver extirpated, he found 1 per cent glucose in the urine. Subcutaneous injection of an aqueous solution of the drug with a small amount of sodium bicarbonate added enabled him to reduce the dose and still prolong its effects. Moritz and Prausnitz (105) learned that the sugar excretion begins three hours following the first of three equal (3.3 grams) doses of phlorhizin at six hour intervals, and the effect is complete in 33 to 36 hours.

In 1895 Coolen (21) proposed the suspension of phlorhizin in olive oil, and this became the standard procedure of later investigators. One gram of the

drug is suspended in 7 cc of olive oil, and the mixture is injected subcutaneously. As the studies continued the D N ratio was accepted as the best measure of effectiveness of the drug. Knopf (69) studied the intensity of glucosuria by changing the amount and method of injecting the drug. One gram of phlorhizin every 8 hours produced a D N ratio of 2.8 to 3.2 during a 10-day period, but the ratio rose to 4.2 during the following days when the dosage was raised to 2 grams three times a day. Minkowski (99) demonstrated that 1 gram daily would produce and maintain a D N ratio of about 2.8 in rabbits. Baer, Hockendorf, Ringer as reported by Lusk (90) obtained similar results. Goldring (51) gave phlorhizin by mouth in some experiments on man and found that the large doses—approximately 400 mgm per kilogram divided into 4 doses at 4 minute intervals—were inadequate to raise the glucose clearance to that of the xylose clearance. He concluded that the oral administration of the drug is much less efficient than intravenous injection. Chassiss, Jolliffe and Smith (19) state that the minimal dosage (intravenous) of phlorhizin required in man to produce total phlorhizinization is found to be in the range of 10 to 20 mgm per kilogram body weight.

As Lusk (91) has stated, the necessary dose for maintaining maximal phlorhizin glucosuria has never been determined but common laboratory practice calls for the injection of 1 gram per day in oil as directed by Coolen (21). In 1941 Weissberger (151) following a recommendation of Sealock, dissolved the phlorhizin in propylene glycol and used this solution for subcutaneous injection with excellent results. The substitution of propylene glycol for the olive oil may prove to be the method of choice of the future. It must be kept in mind that the drug must be pure if one is to obtain uniform results.

*Determination of phlorhizin in body secretions and tissues.* Moritz and Prausnitz (105) reported that the absorption of phlorhizin from the intestinal canal when given per os, is complete. They concluded that the drug is excreted in the urine and that the excretion is complete within 2 days. Loewi (85), however, was able to produce glucosuria by feeding 5 grams of an alcohol extract of a phlorhizinized dog's feces to a second normal dog. Glässner (Lusk 90) produced glucosuria in a normal dog by giving blood from a phlorhizinized dog and extracts of liver and kidney were also found to be moderately effective in this respect. Lambrechts (72, 73, 74, 75) proposed an accurate spectrographic method for the determination of phlorhizin in the serum. He was of the opinion that the kidney was not the principal organ of excretion of the drug since it disappeared from the plasma even when the kidney vessels were ligated. He thought that the muscle could destroy or transform phlorhizin, and that this tissue might be the main site for the elimination of the injected drug.

*Mechanism of action of phlorhizin.* The attention of investigators was early directed towards the effect of phlorhizin on the kidneys because of the glucosuria which develops. However, this abnormality of glucose excretion also directed attention to organs concerned with carbohydrate metabolism such as the liver, pancreas, and other endocrine glands. Many theories have been advanced but none have been proved.

Von Mering (90) proposed his "Elimination Theory" on the basis of the characteristic hypoglycemia that developed and he thought that the elimination of sugar depended on increased permeability of the kidneys for sugar and the excretion took place in the presence of normal or subnormal blood sugar concentrations. He believed that phlorhizin glucosuria was caused by a specific effect of the glucoside on the kidney.

Zuntz (170) first showed that phlorhizin has a direct effect on the kidneys. He inserted cannulas into the ureters of narcotized dogs, and watched the change in sugar concentrations in the urine from the individual kidneys after the injection of phlorhizin into one renal artery. He believed that the glucoside altered the normal retentive power of the kidney epithelium, and the glucosuria was due to secretion of sugar by the kidney cells. This concept was accepted by Erlandsen (40) and other workers of that period.

Levene (82) proposed that the glucosuria was due to increased production of sugar and not a diminished consumption of sugar within the animal, and that the proteins of the body and blood were the source of the extra sugar. This idea of overproduction rather than mere elimination was substantiated in his opinion as he found more sugar in the renal vein than artery, and the quantity of sugar in the kidney tissue increased after phlorhizin injection.

Minkowski (99) advanced his "Vehicle Theory" and expressed it by the equation  $\text{Phlorhizin} \rightleftharpoons \text{Phloretin} + \text{Phlorose}$ . He believed that this breakdown occurred in the kidney. The phlorose (glucose) was eliminated while the phloretin combined with sugar in the blood, and then the new formed phlorhizin split once more in the kidney, etc. This theory was shown to be untenable by Lusk (90) and Charlier (18).

Loewi (85) next proposed the colloidal theory, that is, that part of the sugar in the blood is free and part is bound to a colloid. He considered that in diabetes mellitus, the extra sugar could not be bound by the colloid and was passively excreted through the kidney. In phlorhizin glucosuria, however, the kidney epithelium due to the influence of phlorhizin is enabled to break the colloidal link and eliminate the blood sugar in the absence of hyperglycemia. Stiles and Lusk (143) while accepting this theory added the hypothesis that the colloid sugar cannot be burned. Rosenfeld and Asher (129), Rona and Michelis, and Cammidge (as quoted by John, 66) all demonstrated that the sugar of the blood is in the free state and consequently Loewi's theory is untenable.

Lusk (90) quotes Mosberg and Nussbaum whose experiments were the first to indicate that the action of phlorhizin is on the tubular cells. They showed that the glomeruli of the frog's kidney were nourished by the renal artery, and the tubules by the reno-portal vein. Intravenous injection of phlorhizin was followed by glucosuria even though the glomerular action was cut off by ligation of the renal arteries. Injection of dextrose solution after ligation of the renal arteries produced no glucosuria in the frog until phlorhizin was given whereupon glucose appeared in the urine. In the normal frog intravenous injection of dextrose produced an immediate glucosuria through the glomeruli. Nishu (Lusk, 90) believed that in phlorhizin glucosuria the sugar secretion took



place in the descending parts of the uriniferous tubules rather than through the glomeruli as in other types of glucosuria

Pavy, Brodie and Siau (116) confirmed that no hyperglycemia develops following removal of the kidneys in phlorhizin glucosuria, and confirmed also the work of Zuntz. In their opinion the blood alone gave up sugar to the urine and attributed the glucosuria to an active formation of sugar by a catabolic action of the secretory cells of the uriniferous tubules. The kidneys split the sugar from a closed protein union (Loewi's colloidal theory) and then broke down the protein molecule itself after separation into the nitrogenous compound and sugar. It was assumed that the protein also contained a carbohydrate molecule in its structure. In opposition Coolen (21) found in phlorhizinized rabbits a hyperglycemia which was augmented by extirpation of the kidneys. He did not believe the glucuresis was of renal origin. Erlandsen (40, 41) reported that glucose excretion occurred at normal blood sugar levels and attributed the glucosuria to passage of phlorhizin through the kidneys and characterized it as an actual secretion. He believed that extra-renal effect of the drug had no foundation.

Nash (107) stated there is no doubt about the characteristic hypoglycemia which accompanies phlorhizin glucosuria. Since he found that the renal venous blood in phlorhizinized dogs shows a lower concentration of sugar than the general arterial blood he cast his vote for an increased permeability of the renal epithelium as a cause for the glucosuria. De Boer and Verney (30) agreed with Nash and could not uphold the work of Pavy et al. They used a heart-lung-kidney perfusion preparation and found that the amount of sugar appearing in the urine after addition of phlorhizin to the circulating blood could be completely accounted for by the fall in blood sugar over the same period of time. These results discourage the theory giving sugar producing powers to the kidney.

Häusler (55) postulated that the normal reabsorption of glucose by the tubules is arrested under the influence of phlorhizin and that the impermeability established for glucose normally in the direction of the vessel to the lumen of the urinary tubule is partly reduced.

White (158, 159) presented evidence to show that the action of phlorhizin was one which inhibited the tubules' power to absorb sugar and excited them to excrete sugar. Following this reasoning, Poulsson (119) studied the effects of phlorhizin by comparing the amount of sugar and creatinine, a "no threshold" substance, filtered into the urine, and had to conclude that phlorhizin completely paralyzed the ability of the renal tubules to reabsorb sugar. Shannon et al (135), Shannon and Smith (137), Shannon (136) and Kuhlmann and Deviller (69) performed similar "non-threshold" substance experiments and came to the same conclusion.

Walker and Hudson (149) and Walker et al (148) demonstrated that the greatest amount of glucose reabsorption takes place in the proximal half of the proximal tubule and that the proximal segment absorbs 80 per cent of the glomerular filtrate fluid. They were convinced phlorhizin affected the tubular

reabsorption capacity Walker and Reisinger (150) could find no evidence for ascribing the glucosuria to any change other than the decrease in reabsorption from the tubules

Smith (139) summarizes the kidney function aspect of phlorhizin by stating that "the administration of phlorhizin causes a marked reduction in all renal clearances, presumably due to circulatory disturbances, which fact makes it necessary to judge its effects in terms of clearance ratios rather than absolute values. Phlorhizin produces glycosuria by blocking reabsorption of glucose in all animals. It similarly blocks the reabsorption of xylose and sucrose, the reabsorption of which is slight and apparently due to entanglement with the reabsorption of glucose. The accompanying diuresis is due simply to osmotic resistance offered by the urinary glucose to the reabsorption of water."

Investigations appeared to have reached a standstill with little new work being done to forward the theories of phlorhizin mechanism, until 1933 when Lundsgaard (87, 88) reported his work and presented his phosphorylation theory. He stated that phlorhizin exerted a pronounced arresting effect on phosphorylation and dephosphorylation. He believed that esterification played a decisive rôle in glucose reabsorption, and consequently elaborated his theory of obstruction of phosphorylation by phlorhizin to explain the glucosuria. He considered that the phlorhizin glucosuria was a pure renal phenomenon. Wertheimer (156) verified that selective reabsorption of sugar from the intestines is impeded by phlorhizin and was in accord with reports of Cori (23), and Wilbrandt and Laszt (163). He concluded that the glucoside was a selective poison for the active sugar reabsorption in both kidneys and intestine.

Lambrechts (77, 78, 79, 80) was the first to criticize Lundsgaard's theory since he found, during phlorhizin glucosuria, no general inhibition of the processes dependent on the enzyme, phosphatase. Ellinger and Lambrechts (37) studied the excretion of various dyes in phlorhizinized frogs under the microscope and observed failure of reabsorption in the tubules. This demonstrated that substances other than glucose have their reabsorption affected by phlorhizin.

Anderson and Squires (4), Kutzler and Gutman (71), Weissberger (151), and Beck (8) all brought evidence to disprove Lundsgaard's theory. These papers deal with the study of phosphatase activity and phosphorus metabolism in normal and phlorhizinized animals. All are agreed that there is no evidence to indicate that phosphorylation is affected by phlorhizin. Lundsgaard finally abandoned the theory after he had performed additional experiments according to a report of Walker and Hudson (149).

In summary, none of the proposed theories of phlorhizin action have stood the test of time and experimentation. Von Mering's "elimination theory," Minowski's "vehicle theory," Loewi's "colloid theory," theories of primary renal production of glucose and finally Lundsgaard's "phosphorylation theory" have all been proved to be untenable. Localization of the action of phlorhizin to the epithelial cells lining the proximal convoluted tubules seems fairly definite, and a mass of information concerning renal function has been gathered from these

investigations The glucosuria would appear to be due to interference in reabsorption of the sugar by the kidney tubules, judging from the accumulated evidence

*Anatomical changes in the kidney produced by phlorhizin* Most of the important gross and microscopic changes due to phlorhizin have been recorded by the earlier investigators Lusk (90) quotes the results of several workers Junkersdorf reported hypertrophy of the kidneys if the organism is kept under the influence of the drug for a long period They attain a size comparable to 0.66-1.15 per cent of the body weight, whereas the normal kidney is 0.4 per cent of the body weight Pavy, Brodie and Siau (116) in contrast found no change in the volume of the kidneys Trambusti and Nerti noted necrosis of the epithelium of the convoluted tubules in dogs which received 1 to 5 gram doses of phlorhizin per kilogram for 15 days Von Kossa found severe cloudy swelling of the convoluted tubules of rabbit kidneys, and all the rabbits showed albuminuria Policard and Garnier (117) reported hyaline degeneration of the convoluted tubules of white rats, but no changes in the remainder of the organ

*Phlorhizin effects in nephrectomized animals and those with ureteral ligation* Minkowski (99) noted that the hypoglycemia which developed in phlorhizinized dogs was abolished by extirpation of the kidneys and that the blood sugar remained normal even after the injection of more phlorhizin Schabad (132) showed that ureteral ligation in a phlorhizinized dog caused no change in the blood sugar, whereas in a depancreatized animal marked elevation occurred Csonka (28) observed that after ligation of renal vessels and ureters in a phlorhizinized dog, the subcutaneous or oral administration of 50 grams of dextrose caused an increase in the blood sugar from 58 up to 290 mgm per cent in 2 hours In normal dogs under otherwise duplicate conditions, the increases were only from 75 to 76 mgm per cent

Deuel, Wilson and Milhorat (33) reported that phlorhizinized nephrectomized animals, unlike diabetic animals, were found to be perfectly normal both in the height of the fasting R.Q., and in their response to small amounts of glucose given orally This work showed that phlorhizin exerts no effect on the carbohydrate oxidizing mechanism

Underhill (145) claimed a significant hyperglycemia developed when he gave phlorhizin to dogs which had the renal vessels ligated, or in rabbits in which kidney secretion was abolished as the result of subcutaneous injections of sodium tartrate

Epstein and Baehr (39) found that in the absence of the kidneys, phlorhizin actually stimulated the accumulation of glycogen in the liver The glucosuric effect of the glucoside is abolished as the result of nephrectomy

Nephritis can diminish or entirely stop the sugar secretion in phlorhizin glucosuria Schabad (132), Richter and Haberer (Lusk, 90) damaged the kidney by giving potassium dichromate and noted decrease in sugar excretion with finally cessation when the injury involved all of the organ The normal excretory power of the tubules and of the glomeruli are lost by necrosis of the epithelium, and the phlorhizin, therefore, has no influence

*Effects of phlorhizin on liver* The rôle of the liver in carbohydrate metabolism and its influence on the course of phlorhizin glucosuria are reviewed by Sweet and Ringer (144). Extirpation of the liver in geese with subsequent phlorhizin injection causes no change in the glucosuria whereas it is diminished in frogs (90). Rosenfeld (128) reported that the glucoside produced no glucosuria in Eck fistula dogs, but Sweet and Ringer found such dogs acted like normal dogs in every respect. Pick (Lusk, 90), and Ray, McDermott and Lusk (120) damaged the liver severely by sulphuric acid or by phosphorus and found no alteration in the glucosuria after phlorhizinization.

*Influence on glycogen formation and destruction* According to Von Mering, glycogen persists in the liver of a phlorhizinized animal after a long fast, but Lusk (89) inferred that cold, exercise and adrenalin would mobilize such stores. Ringer (Nash, 110) found extra sugar in treated dogs following adrenalin administration, only if glycogen were present. Anesthesia and convulsions were effective in mobilizing the glycogen. Hoffman (60) was strongly against the idea that phlorhizin could mobilize glycogen from the tissues. Epstein and Baehr (39) found that the body accumulated glycogen in the presence of phlorhizin in the absence of the kidneys. Nash (109) studied dogs with ligated ureters and concluded that phlorhizin did not necessarily affect the glycogen in the liver or muscles. He states, however, that the phlorhizinized dog is able to synthesize and store extensively glycogen while in a state of hyperglycemia, and to a lesser extent even after deglycogenation and a continuing fasting hypoglycemia. Major (93) agreed that glycogenesis occurred when there was hyperglycemia. Schwartz and Sassler (134) reported data to indicate that the liver was disturbed by phlorhizin and that there was interference in glycogenesis.

The relation of glycogen breakdown to production of glucosuria will be discussed later when the general problems of carbohydrate metabolism in phlorhizin poisoning are discussed.

*Effects of phlorhizin on pancreas* Minkowski (99) demonstrated that the glucosuria from phlorhizin could not be due to an effect on the pancreas since the glucoside continued to produce its characteristic effect in depancreatized birds. Nash and Benedict (112) state that the theory of phlorhizin injury to the pancreas was not tenable since *a*, the pancreas shows no alterations, *b*, Zuntz, classical experiment (s. v. p. 257), *c*, phlorhizin has the same effect on depancreatized dogs as on an otherwise normal dog.

Ringer (126) believed that the phlorhizinized dog had two incapacities, the first, an inability to utilize sugar, and second an inability of the kidney to reabsorb the sugar that is filtered from the blood. He believed phlorhizin transiently injured the pancreas since insulin when injected into a phlorhizinized dog caused oxidation of glucose and a concomitant storage of it as glycogen and reduction in the protein metabolism. Nash and Benedict (111, 112) felt that phlorhizin produced a real lesion in the sugar burning mechanism, but that it was not the result of injury to the pancreas as they were able to isolate insulin from the pancreas of maximally phlorhizinized dogs. Colwell (20) also disagreed with the ideas of Ringer.

Cori (22), Nash (108) and Möbius (104) agreed that insulin altered the picture in phlorhizinized animals. Cori noted a decrease in the sugar excretion although the nitrogen excretion was not diminished. Sugar was stored as glycogen which was later excreted when insulin was discontinued. He concluded that insulin did not directly influence sugar formation from proteins during phlorhizin poisoning. Nash reported that glucose and insulin caused abolition or decrease in ketosis and ketonuria, and improved the physical condition of the animal which later became one of extreme prostration when the glucose was discontinued. Extensive synthesis and storage of glycogen occurred in the muscles and liver. Möbius (104) found low glycogen in muscles and liver if phlorhizin was given alone but much higher glycogen contents if insulin was given also.

Dünner and Mecklenberg (36) claimed that insulin is unable to prevent even minute glucosuria brought on after small doses of phlorhizin. However, in diet controlled diabetes they noted a fall in R Q and the appearance of acetone if the glucoside was given. If insulin was given also the R Q rose again and the acetone disappeared. They interpreted these disturbances as due to insufficient sugar combustion in the tissues caused by the phlorhizin.

Allen (1) as a result of sugar tolerance studies concluded that phlorhizin does not injure the islets of Langerhans. Goldstein et al (52, 53) analyzed glucose tolerance curves obtained from intact dogs and from dogs with the ureters ligated when insulin was given as well as phlorhizin. They concluded that insulin exerts its entire effect on the sugar utilization and that it in no way corrected the action of phlorhizin on the kidneys.

As a result of all of this work, it would appear that phlorhizin has its effects on the kidney and that no injury is caused to the pancreas.

*Effects of phlorhizin on hypophysis* Houssay and his co-workers (11, 61, 62) noted that the phlorhizin glucosuria of a hypophysectomized dog was characterized by *a*, high mortality, *b*, elimination of glucose less actively, *c*, diminution of urinary nitrogen, and *d*, low D N ratios. With a lesion of the tuber cinereum the glucosuria is identical with that of controls, indicating a dissociation of function of this part of the gland. Injection of extract of the anterior lobe of the pituitary in hypophysectomized dogs is beneficial since it *a*, prevents the hypoglycemia and death, *b*, increases the glucosuria to control levels, *c*, increases the diuresis and fall in weight, *d*, increases ketone body elimination, but *e*, does not always increase the urinary nitrogen.

Rietti (122) found that ketone excretion of phlorhizinized dogs is much less than that of normal controls and that pituitary insufficiency always diminished the urinary elimination of ketone bodies. Black (13) reported that rats treated with anterior pituitary extract containing the ketogenic principle became resistant to its action within 3 months and such animals showed only slight acetoneuria when treated with large doses of phlorhizin while fasting.

Gaebler and Zimmerman (47) gave a large injection of the anterior pituitary growth hormone to a phlorhizinized dog on the fourth day and reported a weight increase and a smaller nitrogen output than in experiments with phlorhizin.

alone The effect of this preparation used above on nitrogen output was increased by thyroidectomy, presumably because the absence of the gland reduces the metabolic rate of phlorhizinized dogs to approximately normal levels In thyroidectomized-depancreatized dogs receiving a constant amount of insulin, the same doses of the pituitary extract were severely diabetogenic

*Effects of phlorhizin on adrenals* Adrenalectomy in phlorhizinized rats greatly decreases sugar and nitrogen excretion according to Evans (43) Adrenal cortical extract does not alter the sugar or nitrogen excretion of adrenalectomized phlorhizinized rats Ketosis of glucoside treated rats is diminished by adrenalectomy He concluded that the adrenal cortex is concerned in the conversion of protein to carbohydrate

Robbers and Westenhoeffer (127) report that the glucosuria of phlorhizin is not influenced by adrenal cortical extract or corticosterone Wells and Kendall (153) were able to increase the low rate of glucose excretion of the phlorhizinized, adrenalectomized rat by giving corticosterone and closely related compounds The rate of gluconeogenesis of operated rats could be increased to that of normal phlorhizinized rats by the compound E (17 hydroxy 11 dehydrocorticosterone) This finding illustrates the importance of the adrenal cortex in carbohydrate metabolism

Wells and Chapman (152) determined that in hypophysectomized phlorhizinized rats the excretion of glucose and nitrogen was about half that found in adrenalectomized-phlorhizinized rats Treatment of such rats with compound E or with corticosterone acetate markedly increased the rate of gluconeogenesis If such rats were given compound E and thyrotropic hormone the rate of gluconeogenesis approached that of normal phlorhizinized rats Thus, both the adrenal and thyroid glands affect the formation of glucose in hypophysectomized-phlorhizinized rats

Wells and Kendall (154) state that results obtained when the adrenal medulla only is removed, indicate that the action of epinephrine is not significant These workers conclude that the metabolism of exogenous protein is not seriously impaired by the absence of the adrenal cortical hormone It is only where the exogenous supply is lacking and endogenous protein must be metabolized that the limited capacity of gluconeogenesis of the adrenalectomized animal becomes evident

*Effects of phlorhizin on thyroid* Dann et al (35) reported that thyroidectomy may lead to failure of increase in heat production usually observed after phlorhizin administration and protein metabolism is affected also Wills and Kendall (153) noted that thyroidectomy diminished the amount of glucose and nitrogen excreted by phlorhizinized rats but that thyroxine restored the rate of gluconeogenesis

The effects of phlorhizin on secretions of the breasts, skin glands, and on pancreatic, gastric and salivary secretion have been studied (26, 118, 90, 110) The most that can be reported is that the secretion may contain a reducing substance in very small amounts Splenectomy does not effect phlorhizin glucosuria (5)

*Influence of phlorhizin on body fluids and secretions* Von Mering noted that the composition of the urine of a phlorhizinized dog resembled that of a human diabetic. There was a high ammonia content, much acetone, and a strong levo-rotation after fermentation, indicating the presence of B-hydroxy-butyric acid. The dog finally became comatose from which state it could be aroused by giving meat and milk by stomach tube. He also noted that after giving phlorhizin to a starved dog the amount of nitrogen in the urine was increased, but this increase was not as large if fat were fed at the same time. Moritz and Prausnitz (105) discovered that the absolute amount of sugar excreted from meat and carbohydrate food depends on the ingested amount. As much and more sugar can be obtained from the meat as from carbohydrate if the meat is given in abundant proportions. Coolen (21) confirmed these findings.

Reilley, Nolan and Lusk (121) observed that a starved phlorhizinized dog excreted five times the amount of nitrogen as that excreted by an ordinary fasting dog. The maximal nitrogen excretion appeared on the second or third day of phlorhizinization and fell gradually.

Loewi (Lusk, 90) reported that diuretics in normal kidneys increased the secretion of sodium chloride and urea, but not  $P_2O_5$  and if given during phlorhizin glucosuria there is an increase in the chloride secretion but not in the secretion of  $P_2O_5$  or dextrose. He concluded that chlorides, urea, and sugar are easily filtered through the glomeruli, while  $P_2O_5$  is freed from combination in the blood and preserved by the kidney.

Lusk (90) reported that urea nitrogen in a starved dog was 89 to 92 per cent of the total nitrogen. Excreted ammonia rose sharply and in one case reached a maximum on the second day of the glucosuria which was the same day the maximum acetone and B-hydroxy-butyric acid excretions occurred. Creatine nitrogen increases were also noted. Sulfur content rose proportionally to the rise of nitrogen, thus maintaining the ratio found in the fasting condition.

Mandel and Lusk (94) studied the carbon in the urine and reported that as much as 60 per cent of the phlorhizin carbon may be eliminated in the urine. In the early stages of phlorhizin glucosuria the carbon in the urine derived from oxybutyric and other abnormal products except sugar appears to be negligible.

*Effects of phlorhizin on blood* The hypoglycemia first noted by Von Mering is the most important alteration in the blood of the phlorhizinized organism. Erlandsen (40) showed that the blood sugar level depended on the amount of glycogen in the liver available for use as glucose. De Boer and Verney (30) demonstrated that there was a direct relation between the amount of sugar appearing in the urine and the fall of the blood sugar during the same period.

Ets (42) found an acidosis, as indicated by the fall in alkali reserve and increase in  $H^+$  ion concentration, hypoglycemia, an increase in the blood N.P.N., and an increase in the blood lipid constituents.

Himwich et al (58) observed that the liver of phlorhizinized dogs removed fat from the blood until the fifth day when the accumulated fat began to be liberated. In depancreatized dogs continuous accumulations of fat from the

blood were noted. They (59) also studied the blood in the portal and hepatic veins in phlorhizinized dogs and learned that acetone is added to the blood by the liver.

Levene (81) found decreased sugar in the lymph and also thought there was sugar in the bile as the result of phlorhizin injections. Ray, McDermott and Lusk (120) discovered the presence of bile salts but no traces of sugar in the vomitus of phosphorus poisoned phlorhizinized animals, but Woodyatt (165) reported evidence of glucose in bile.

Smyth and Whipple (140) determined that phlorhizin has no effect on bile salt output in bile fistula dogs.

*The D N ratio* An excellent review of the D N ratio is given by Lusk (90) and the essential details only are given below. Minkowski found in his de-pancreatized dogs that the urine constantly contained dextrose and nitrogen in a ratio which approached 2.8 grams of dextrose to 1 gram of nitrogen. Since 1 gram of nitrogen is equivalent to 6.25 grams of muscle protein, it was calculated that in "total pancreatic diabetes," 45 per cent of the protein molecule was excreted in the urine as dextrose.

Lusk originated the term "extra sugar" to indicate the sugar which resulted from an ingested substance or from the carbohydrate catabolized in the organism itself, and not from simultaneous protein metabolism. The sugar contained in the glucoside phlorhizin does not affect the D N ratio.

Cremer and Rutter after giving frequent doses of phlorhizin to fasting rabbits obtained ratios varying between 2.64 and 2.8 and considered such ratios to indicate "total phlorhizin diabetes." Reilley, Nolan and Lusk were unable to produce a ratio of 2.8 in dogs but established ratios in a range 3.38 to 3.91. Later corroboratory work confirmed such ratios and consequently the customary D N ratio in a fasting phlorhizin dog is considered to be 3.6 to 3.7. Lower ratios are seen terminally in dogs which die of phlorhizin poisoning or some other pathological lesion.

On the basis of a 3.65:1 ratio of glucose to nitrogen, it is calculated that about 58 per cent of the protein molecule is convertible to sugar. Reilley, Nolan, and Lusk (121) concluded that when meat is fed, the sugar derived therefrom is quantitatively recovered in phlorhizin glucosuria, but it may be eliminated before the nitrogen belonging to it. Nash and Benedict (111) confirmed the different excretory rates of dextrose and nitrogen.

*Influence of body glycogen* An especially high value for the D N ratio will always be found in the urine of the first day of a fasting phlorhizinized dog. The excess of sugar is due to the loss of blood sugar and from mobilization of glycogen stores. Phlorhizin alone does not clean out all of the glycogen from the body, but cold, work or adrenalin administration during phlorhizinization are reported to cause complete freeing of the tissues of their glycogen stores. However, correct ratios of 3.6 to 3.7 may be obtained by fasting and phlorhizin administration over a period of 3 to 4 days.

Lusk (90) concluded as the result of many experiments that phlorhizinized



animals store up no glycogen and that when sugar is formed from ingested material, all of it will be eliminated and none stored as glycogen. He concluded also that sugar cannot be produced from fat.

*Influence of carbohydrate feeding* Dextrose fed in small quantities per os or injected subcutaneously is not utilized but eliminated quantitatively. Phlorhizin glucosuria is considered to be a complete glucosuria and dextrose (within limits) cannot be utilized (143).

*Influence of protein feeding* Reilly, Nolan and Lusk (90) analyzed the effect of meat feeding on the D N ratio. They noted that after feeding 500 grams of meat the nitrogen and sugar excretion in the urine can be doubled while the D N ratio remains unchanged. The sugar arising from the protein can be excreted more quickly than the corresponding nitrogen. Gelatin feeding also causes no change in the ratio but the protein metabolism of the body was decreased.

*Influence of fat feeding* Fat feeding in phlorhizin glucosuria has no effect on the sugar excretion in the sense that the ingested fat is not changed into dextrose. Von Mering showed that fat ingestion reduced the nitrogen and sugar excretion in the urine. Reilly, Nolan and Lusk (90) established that fat feeding had no effect on the D N ratio.

*Acetone bodies* While the fasting dog shows almost no acidosis, the fasting phlorhizinized dog secretes acetone and B-hydroxy-butyric acid in the urine and coma finally develops.

Various investigators (48, 10, 160, 161) have demonstrated that meat or carbohydrate feeding to a phlorhizinized dog lessens the degree of acidosis with reduction in the amount of acetone and hydroxy-butyric acid. Study of the D N ratio in the urine showed that the fall in acidosis cannot be accounted for by combustion of the sugar.

The acetone content of the blood, liver, kidneys and lungs from a phlorhizinized dog may be double the amount found in a normal dog, and it has been demonstrated that B-hydroxy-butyric acid is oxidized when given to a normal dog but very poorly oxidized by a phlorhizinized dog (90). Himwich et al (59) by determinations on the portal and hepatic venous blood found that acetone was added to the blood by the liver. Feeding of the sodium salt of B-hydroxy-butyric acid does not effect any change in output of sugar by a phlorhizinized dog (106).

Soskin (141) postulates two theories of ketosis and the formation of the ketone bodies *a*, that an inability of extra-hepatic tissues to dispose of the ketone bodies exists, *b*, a rate of hepatic production in excess of the existing normal rate of peripheral disposal is present. Nash (109) was of the opinion that the acidosis and ketosis which characterize phlorhizin glucosuria may result from the lowered concentration of carbohydrate in the tissue.

Mirsky (100) demonstrated that ketones arise only in the liver, and that insulin which suppresses ketosis does so by acting on the liver and not on the extra hepatic tissues. Evidence indicates that in diabetes (pancreatic) the ketone bodies, like the blood sugar, are utilized by the extra-hepatic tissues, but are produced by the liver at rates which exceed the power of the tissues to dispose

of them Mirsky and Brohkahn (102) attributed the effect of glucose in ketosis to an inhibition of fat oxidation in the liver. Ketosis depends on the rate of ketone formation and the rate of ketone utilization.

Möbrus (104) found that under the influence of insulin, the sugar and nitrogen excretion in the urine declines in the phlorhizinized fasting animal. Insulin prevents the ketonuria that is characteristically present.

According to Rietti (122) ketone elimination was less in pancreatic diabetes or hypophysectomized animals than in controls, and was related to the level of the blood sugar. In fed hypophysectomized dogs, the ketone elimination in phlorhizin glucosuria is always less than in corresponding controls. Sugar feeding diminishes the ketone excretion in the controls, but in hypophysectomized animals there is a slight rise. Ketone excretion of phlorhizinized-hypophysectomized animals does not increase as much as does the glucosuria on a meat diet.

Goldfarb and Himwich (50) studied the ketone substances in depancreatized and phlorhizinized dogs. In 8 dogs the heart removed acetone substances from the blood and in 3 cases added them, but the brain caused no changes in the blood. The testicles removed acetone in 6 cases and showed no change in 8 others. These results were interpreted as giving some indication of the character of the foodstuffs oxidized by the individual organs.

*Total and intermediary metabolism.* Experiments concerning the total energy metabolism of phlorhizinized animals are adequately covered in Lusk's review (90).

The rôle of phlorhizin glucosuria in the study of intermediary metabolism constitutes one of the most important aspects of scientific investigations with this drug. While phlorhizin glucosuria is essentially an interference with metabolism of carbohydrates, much new light has been thrown on the close interrelationship of the three major groups of nutrient material, carbohydrate, protein and fat, by the use of the drug.

Phlorhizin is not only the most convenient but the more effective means of inducing experimental diabetes, in respect to the degree of carbohydrate intolerance created. Early experiments on the carbohydrate intermediary metabolism are summarized by Lusk (90). When *levulose* or *galactose* is given to a completely phlorhizinized animal, some extra dextrose appears in the urine but no levulose or galactose is excreted. No lowering of the protein metabolism was noted (90, 121). When *lactose* is fed, Lusk reported that no lactose was present in the urine but the protein metabolism was markedly reduced, and the reabsorption and combustion of a large part of the lactose was demonstrated. Following the intravenous injection of *sucrose* the sucrose secretion increases without extra dextrose appearing in the urine. If an equimolecular solution of dextrose and sucrose are given intravenously, the phlorhizin effects a greater excretion of sucrose than of dextrose. Pentoses and cellulose caused no increase in dextrose excretion in the urine. Glycerin, lactic acid, and propyl alcohol are changed into dextrose in the phlorhizinized organism, while other alcohols and both mono- and di-basic fatty acids have no influence on the D/N ratio and are not converted into dextrose (90).

Baer and Blum (7) reported that subcutaneous injections of glutaric acid in

a phlorhizinized dog considerably reduced the dextrose, the nitrogen, and the B-oxybutyric acid of the urine. However, they also stated that glutamic acid, alanine, glycol and lactic acid had no remarkable influence on the amount of excreted sugar, and it is well known that these substances are sugar formers. Lusk (90) considered that their animals were incompletely phlorhizinized and that conclusions drawn from the experiments were not conclusive.

Mandel and Lusk (95) noted that lactic acid disappears from the blood and urine in phosphorus poisoning when phlorhizin glucosuria is induced. They concluded that this indicates that lactic acid, produced from cleavage and denitrogenation of protein, is first synthesized to dextrose before distribution to the tissues.

Loebel et al (84) found that lactic acid may be formed in an animal which has lost the power to oxidize carbohydrate and present this as evidence that the defect in phlorhizin glucosuria is not in the formation of lactic acid from carbohydrate. This work contradicts Embden (38) who suggested that carbohydrate could not be oxidized in phlorhizin glucosuria because it could not yield lactic acid. Loebel et al hypothesized that during the contractile phase of muscular activity energy is supplied by the exothermic chemical reactions which result in the formation and neutralization of lactic acid. These reactions occur even during complete phlorhizin glucosuria and glycogen depletion. During the recovery phase the lactic acid is reconverted to glycogen and they thought that in phlorhizin glucosuria the chief, if not the only, source of energy during the recovery phase must be furnished from fat.

The conversion of succinic acid to glucose in the phlorhizinized dog is reported by McKay and Barnes (92) in the ratio of 2 mols succinic acid  $\rightarrow$  1 mol dextrose. When larger doses are given, a small percentage is excreted in the urine as sugar, but anti-ketogenic and nitrogen sparing activity indicate its conversion to dextrose before being burned.

Deuel and Chambers (32) determined the hourly rate of elimination following the oral administration of glucose, galactose, fructose and lactose in phlorhizinized dogs. Rate of excretion of fructose and galactose were practically identical with that of glucose itself, thus indicating that the intermediary transformations involved in the change of these monosaccharides to glucose is a very rapid one. Ninety-four per cent of the fructose and 80 per cent of the galactose was recovered as extra glucose but lactose was more slowly excreted and 50 per cent only was recovered in 2 hours as dextrose. Sparing action of nitrogen metabolism was noted despite the fact that none of the sugar was apparently burned. Muscular power of the glucoside treated animal was restored even though the glucose was quantitatively excreted.

The major problem in the intermediary metabolism of carbohydrate is *whether or not the glucose is actually utilized by the organism in the oxidative energy-producing process*.

Lusk (90) stated that if the phlorhizin glucosuria is a "total glucosuria," then the ingestion of dextrose must be followed by a complete excretion in the urine in the form of extra sugar. Stiles and Lusk (143) believed that within certain

limits the phlorhizinized dog had lost its power to oxidize dextrose but if carbohydrate is given in sufficient amounts, it can be oxidized

Rosenfeld (129) gave a normal dog 100 grams of dextrose by vein and recovered 21 to 28 grams of extra sugar in the urine, whereas 36 grams appeared in the phlorhizinized dog. If the dextrose was fed, he recovered 78 grams of extra sugar. Lusk attributed these findings to the degree of concentration of dextrose in the blood stream. If intravenously given, the sugar concentration in the blood stream would be high and cells would obtain it in amounts sufficient for oxidation and less elimination would result. When fed, absorption would be slower and consequently concentration would be much less and so it was not oxidized and was recovered as such in the urine. Sweet and Ringer (144) express the findings of Rosenfeld in a formula, viz. The amount of glucose that may be oxidized is a function of the ratio between the velocity of absorption of glucose and the velocity of its excretion by the kidneys.

Nash and Benedict (111), however, produced hyperglycemia which was maintained for 6 hours or longer but quantitatively recovered the sugar in the urine. They took the view that phlorhizin produces an intrinsic impairment of utilization of sugar by the tissues. Wiersuchowski (162) also considered that there was a general hindrance in glucose utilization since phlorhizin treated animals die as the result of hypoglycemia with convulsions or ketosis with coma. Both of these states are visible exponents of the extremely low level of carbohydrate metabolism in the body cells.

Deuel, Wilson and Milhorat (33) working with nephrectomized phlorhizinized animals found that the nitrogen metabolism was like that of controls which received no phlorhizin. Acetone was not produced as in the ordinary animal, and blood sugar values were constant. The phlorhizinized nephrectomized dogs must have oxidized glucose normally. They suggested that two avenues of escape from oxidation are offered the ingested glucose. The first is that a large proportion is rapidly removed from the field of possible oxidation by excretion as extra sugar, and second, that it is stored as glycogen temporarily which is later excreted when the blood sugar falls. The evidence indicates that the action of phlorhizin is entirely a renal one. Carbohydrate is not oxidized during fasting or following ingestion of small amounts of glucose since the concentration of glucose in the blood and tissues is low and not due to any intrinsic impairment in the mechanism for its oxidation.

Boothby et al (15) studied the respiratory quotient and D/N ratio in phlorhizinized dogs and discovered that the basal metabolic rate is markedly elevated and may reach 90 per cent above the normal value. Administration of glucose causes a definite decrease in the high level of heat production and a definite rise in the R/Q. If the rise in R/Q is taken as indicating an increase in the oxidation of glucose, it is found that 18 to 25 per cent of the ingested glucose is oxidized in 5 to 7 hours.

Deuel (31) also presented evidence indicating that there is no intrinsic impairment of the ability to oxidize carbohydrate existing in the phlorhizinized animal but that phlorhizin action on the kidneys results in mobilization and

drainage of available glucose The carbohydrate ceases to be oxidized because of the low concentration in the blood and tissues and there is no stimulus for insulin production Acidosis ensues and the glucose tolerance is reduced The glucose tolerance curves of the phlorhizinized animals are distinctly of the diabetic type

Drury, Bergman and Greely (34) studied phlorhizinized hepatectomized dogs and determined that 75 mgm per hour of glucose are required to maintain such an animal As a result of their experiments, they were convinced that the tissues of such glucoside treated dogs do utilize sugar even though the blood sugar level is kept low Bollman, Mann and Magath (14) concluded that the energy turnover of the liverless animal does not come from protein but rather from the *direct utilization of fat*

Soskin, Levine and Lehmann (142) concluded also that there is no diminution in the utilization of carbohydrates by phlorhizinized dogs as compared with normal dogs They believe the low R Q in phlorhizin glucosuria is due to overproduction rather than under-utilization of sugar The low R Q results from a *predponderance of reaction in which oxygen is used up without the corresponding production of carbon dioxide*, such as the formation of sugar from protein and fatty acids The excessive gluconeogenesis in both phlorhizin glucosuria and pancreatic diabetes, although caused by different mechanisms, nevertheless lead to similar consequences, namely, continued glucosuria, low liver glycogen values, accumulation of fat in the liver, ketosis and a lowered R Q

It is therefore apparent that the D N ratio of phlorhizin treated animals cannot signify that no glucose is being utilized, or that sugar which is excreted represents either the partial or complete amount which is being formed from protein alone

Shorr, Loebel and Richardson (138) present experiments with both living rats and excised rat tissue Excised renal tissue from normal rats and from phlorhizinized rats responded to the presence of glucose by an increase in oxygen consumption and corresponding rise in the R Q The average R Q 0.850 indicated that carbohydrate was furnishing about 50 per cent of the energy This work is against the theory that phlorhizin interferes directly with the oxidation of carbohydrate by the cells in general Bach (6) reported that carbohydrate synthesis in liver slices is partly suppressed by sodium fluoride and iodoacetate, but is apparently increased in the presence of phlorhizin

Fleischmann (44), however, from the study of excised tissues believed that phlorhizin blocked the mechanism for sugar absorption in the body as well as the tubular epithelium of the kidney Wetzel and Zitzewitz (157) report a rise in the R Q of slices of liver and diaphragm put in Ringer's solution containing 0.2 per cent glucose This phenomenon is completely inhibited by phlorhizin

The consensus of opinion is that there is no essential defect in the utilization of sugar caused by phlorhizin but the lack of utilization is due to its very rapid loss through the kidney

*Intermediary metabolism of protein* Loewi (85) first showed that amino acids originating from the proteolysis of meat could maintain nitrogen balance

even as protein itself. Stiles and Lusk (143) fed a dog a meat pulp which had stood 14 months with trypsin proteolysis and concluded that prolonged digestion destroyed one of the principal dextrose producing substances so that only a 40 per cent conversion of the ingested materials into dextrose resulted.

It has been shown by various workers (90, 125) that glyocol and alanine are completely converted to dextrose and that three of the four carbon atoms of aspartic acid and three of the five carbons of glutamic acid are changed to dextrose. Tyrosin had no influence on sugar excretion. Propionic acid (124) is completely converted to glucose, and methionine (146) is also a sugar former.

Dakin (29) in 1913 summarized the data relating to glucogenetic properties of the amino acids as recognized at that time. Serine, cystine, proline, ornithine and arginine are all capable of yielding large amounts of sugar when given to glucosuric dogs. Valine, leucine, isoleucine, lysine, histidine, phenylalanine and tryptophane yield little or no sugar. He concluded that amino acids derived from protein which may yield glucose freely are all those containing two, three, four and five carbon atoms with the exception of valine. Arginine is the only amino acid with more than five carbons which may furnish glucose freely. All straight chain amino acids yield sugar except lysine while the amino acids with branched chains, including valine, leucine and isoleucine furnish little or possibly no sugar. Proline is the only cyclic amino acid yielding much glucose. None of the aromatic amino acids yields glucose in considerable amount.

Corley (24, 25) reported that  $\beta$ -alanine and  $\epsilon$ -amino caproic acids are not sugar formers but  $\gamma$ -hydroxybutyric acid is a sugar former in the phlorhizinized animal. Hydracrylic acid,  $\delta$ -hydroxyvaleric acid and  $\epsilon$ -hydroxycaproic acid produced no sugar. He believed these results indicated that successive stages in the breakdown of the diamino acids may result in the acids with one less carbon atom, with an amino group in the  $\omega$ -position, the corresponding  $\omega$ -hydroxy acids, and the corresponding dicarboxylic acids.

Janney (64) discovered that each protein produces a definite amount of glucose in the phlorhizinized organism. The various yields represent 50 to 80 per cent by weight of the protein fed. These yields approximate the ratios which the glucogenetic amino acids of the protein in each case bear to the total amino acids as determined by hydrolysis.

The extra glucose in phlorhizinization is derived from the protein fed rather than from the fats, glucose or protein of the body (61). This is indicated by the fact that glucose fed to phlorhizinized dogs in the same amounts as the extra sugar excreted after feeding glucogenetic substances reappears quantitatively in the urine. Administration of substances incapable in view of their chemical structure of being converted into glucose has been found not to be followed by extra glucose formation. In case of ingested proteins, the extra glucose elimination rises and falls nearly parallel with an extra nitrogen elimination. It is evident that dextrose must be considered one of the chief intermediary products of protein metabolism.

Mirsky, Heman and Swadesh (103) report on the nitrogen sparing action of glucose. The rate of nitrogen metabolism is greater than that of the normal in

both phlorhizinized and completely depancreatized dogs, and intravenous glucose in large amounts causes lowering of this high rate of protein metabolism.

Gray, Ivy and Cuthbert (54) fed casein to phlorhizinized dogs and depancreatized dogs and came to the conclusion that such animals give low glucose yields from ingested protein and suggested that there was impairment of gluconeogenesis. They confirm the significant difference between the D/N ratio obtained during fasting experiments on depancreatized and phlorhizinized dogs.

Janney (64) confirmed the earlier findings that the glucose urinary excretion curve reaches its peak before the nitrogen curve. It is possible that the greater permeability of the phlorhizin poisoned kidney for glucose may explain this phenomenon. Csonka (27) found that the rapidity of absorption and elimination of ingested glucose by the phlorhizinized dog is almost the same as the rapidity of the absorption of isoglucogenic quantities of glycocoll or alanine and of the elimination of the synthetic sugar produced from them after deamination. The amount of extra sugar elimination in phlorhizin glucosuria appears to be a better index of the intensity of the hourly metabolism of the amino acids than does nitrogen output under these conditions.

Lusk (90) noted that protein catabolism in phlorhizin glucosuria is increased very greatly above the starvation requirement. Ringer (123) analyzed the whole protein metabolism into three components: 1, minimal nitrogen requirements, 2, dextrose nitrogen, 3, complementary nitrogen. Nitrogen of no. 1 can be replaced only by protein, nitrogen of no. 2 can be spared by a sufficient supply of carbohydrates (50 per cent of the caloric requirements). It cannot be spared by fat. Nitrogen of no. 3 can be spared by any foodstuff.

Experiments support Landergren's theory "that in starvation a certain fraction of protein is metabolized for the formation of glucose, and this fraction can be spared by carbohydrates and fat."

If protein metabolism is dependent to a certain extent on the concentration of glucose in the blood and body fluids, then the feeding of glucose to phlorhizinized dogs in proper quantities ought to cause a lowering in protein metabolism, while in depancreatized animals the sugar should have no effect on it. Ringer concluded that in phlorhizin glucosuria the protein metabolism rises in part because of the hypoglycemia and that the giving of glucose did spare protein even though all the glucose given was eliminated and none of it was burned.

Chambers and Lusk (17) found the S.D.A. of glycine as measured by its calorogenic action, to be dependent on the amount of glycine administered and to be independent of the size of the animal which received it.

Lusk (89) reported that the influence of work, sufficient to double the fat metabolism, has no effect on the D/N ratio. A rise in protein metabolism, as indicated by the urinary nitrogen findings, accompanied the mechanical work only when extra carbohydrate was also eliminated.

Soskin (141) in a review of the blood sugar and factors affecting it, states that there is no factual basis for concluding that phlorhizin alters the bio-chemical processes in such a manner as to allow a larger proportion of the protein molecule to be converted into sugar. And, if a constant proportion of the protein molecule is convertible, then either the depancreatized animal always utilizes a

significant fraction of the sugar derived from protein, or the phlorhizinized animal must be forming sugar from fat as well as protein

Biasotti and Houssay (11) (s v p 262) noted that in hypophysectomized-phlorhizinized dogs on a meat diet, the loss of weight and glucosuria are much less than in normal controls. They believed that the capacity to form sugar at the expense of the endogenous protein was thus greatly diminished by hypophysectomy.

Howland and Hawkins (63) fed plasma protein by mouth to fasting phlorhizinized dogs and noted that it was ingested with partial conversion to sugar. When injected intravenously, the protein disappeared promptly from the blood stream. No protein was lost and no excess elimination of sugar and nitrogen in the urine occurred, and nitrogen conservation was apparent in the after period. They postulated that the animal did not have to break the protein down to amino acids before utilization of it but rather that the protein was partially catabolized to larger protein aggregates and these reassembled to form the desired type of tissue protein. Hawkins and his co-workers, unpublished data, administered a mixture of four sugar forming amino acids by mouth and by vein to glucoside treated dogs. This mixture was partially broken down with increase in sugar and nitrogen excreted in the urine. As would be expected, the extra sugar and nitrogen recovered was the same whether the mixture was fed or given parenterally. When casein digest was given by mouth, it was partially converted to sugar and nitrogen with resulting increase of these substances in the urine. A similar reaction occurred when it was given intravenously, but more conservation was noted in the after period. This suggests that the digest when given parenterally is more effectively utilized under the conditions of the experiment. These data confirm the value of casein digests as an intravenous food and potential tissue repairing and replacement agent.

*Intermediary metabolism of fat* It is long since a matter of experimental demonstration that fat may be formed from carbohydrate and hence from protein. It is believed that carbohydrate does not form from fat. Fat feeding even in excess of the total energy requirements is without effect on the D N ratio of phlorhizinized animals. Work capable of more than doubling the fat metabolism has no effect on the sugar output of the completely diabetic organism. These facts indicate according to Nash (110) that there is no doubt that fat cannot be converted to sugar.

Lusk (89) found that phlorhizinized fasted dogs exposed to cold and mechanical work showed great increase in their fat metabolism, but there was no increased sugar production as the result of the fat combustion.

Hawley (56) studied dogs with fatty livers or fat-fed dogs and could find no evidence of gluconeogenesis even when conditions were such that the animal needed carbohydrate. These dogs were given insulin in addition to the phlorhizin. Page and Young (115) injected phosphotide 100 cc of a 10 per cent aqueous brain emulsion, intravenously and found no appreciable alteration in the urinary D N ratio in glucoside treated dogs. This is further evidence against the conversion of fat into sugar.

*Mineral metabolism* The effect of phlorhizin on the mineral metabolism of



the body is a comparatively recent subject and was studied originally as a result of its close relationship with some of the more obvious phenomena of phlorhizin glucosuria. Nash (108) noted, in close parallel to the repression of the ketosis of fasting phlorhizinized dogs following glucose ingestion, there is a sharp drop in the inorganic phosphorus of the blood. Coincidentally, the inorganic phosphorus of the urine diminishes rapidly and most frequently disappears entirely. A definite renal threshold for phosphate is indicated. The larger the dosage of sugar, the more pronounced and prolonged the effect on the urine phosphates. During this period of heavy sugar excretion, the urine reaction changes from strongly acid to faintly acid or neutral. Harrop and Benedict (quoted by Nash, 110) suggest that the phosphate withdrawn into the tissues, as noted in normal animals, is concerned with an intermediary stage in the storage of carbohydrate as glycogen, which occurs in phlorhizinized animals. It may be that the "alkaline tide" after meals is a reflection of such a process. The prompt recovery of the alkaline reserve in the phlorhizinized dog after dextrose feeding may not be due entirely to the burning of ketone bodies. It is possible that the elevation of the carbon dioxide combining power of the blood in human diabetics after insulin administration likewise may be largely attributable to glycogen synthesis and the withdrawal of blood phosphates. Henderson (57) presents the idea that the condition chiefly determining the extent of this withdrawal of alkali, or its return to the blood, is the freedom with which sugar is oxidized in the tissues.

Phlorhizin given for 7 days to dogs on a constant diet of lean beef by Kastler (67) produced a depression of potassium and an elevation of serum inorganic phosphorus in the blood. Sodium, calcium, magnesium and chlorine in the whole blood remained unchanged, as did the NPN. In spite of continued weight loss by the animals, the potassium and phosphorus values returned to normal in the after period. There was no compensating change in the potassium or phosphorus in the urine during either the phlorhizinization or the after periods that was not accounted for by slightly increased protein catabolism.

Lambrechts (78) found that phlorhizin raises the threshold of the renal excretion of phosphorus, while it lowers that of glucose.

Yamada (168) reported the total phosphorus of the blood to be decreased markedly by epinephrine, phlorhizin and glucose administration.

Ziegler and McQuarrie (169) found effects of phlorhizin alone on the blood constituents similar to those of Kastler (67), except for the absence of a significant increase in the serum inorganic phosphorus. While the serum potassium was regularly reduced by phlorhizin alone, it remained unchanged when phlorhizin administration was accompanied by the ingestion of potassium chloride in large amounts. On the other hand, the reduction of serum potassium as a result of phlorhizin administration was apparently accentuated by the feeding of excessive amounts of sodium chloride. In neither instance, however, did the extra salt ingested significantly influence the degree of phlorhizin hypoglycemia. The glucosuria due to phlorhizin administration was likewise essentially unaffected by either salt. The slight increase observed in some experiments was obviously due to accentuation of the phlorhizin diuresis by the salt ingested.

Slight variations, which were noted in the excretion of nitrogen and of minerals, could be accounted for in the same way. The calcium, magnesium sodium, NPN, chlorides and carbon dioxide content of the serum were not significantly altered by the procedures employed. The conclusions from these experiments are that the carbohydrate metabolism due to phlorhizin poisoning bears no relationship to that of diabetes mellitus as regards its response to ingestion of excessive amounts of sodium and potassium salts. The characteristic effects of these salts on hepatic glycogenesis in the normal dog appear to be abolished by phlorhizin.

McQuarrie, Thompson and Anderson (96) found that feeding large amounts of sodium chloride increased the ability of diabetic children to utilize carbohydrate as shown by the marked decrease in fasting blood sugar and glucosuria. Potassium salts had a diametrically opposite effect, one part potassium counteracting the effect of three chemically equivalent parts of sodium.

*Phloretin*. It is generally observed that phloretin, which, with phlorose (glucose) constitutes the products of the acid hydrolysis of phlorhizin, does not act as strongly as phlorhizin. Ringer (quoted by Lusk, 90) gave a fasting dog 10 grams of phloretin by mouth without producing a glucosuria and found a remarkable glucosuria only when the phloretin was given with meat. This indicates that digestive activity is necessary for the resorption of phloretin.

Lusk (90) gave phloretin and dextrose subcutaneously at the same time to rabbits without producing a glucosuria. From this he inferred that the subcutaneous tissue does not have the ability to reconstruct phlorhizin from its two components.

Lambrechts (76) prepared synthetic phloretin to test its glucosuria producing properties. He verified the purity of his product by his spectrographic method (72) (p. 256). He used several other phlorhizin derivatives, namely, phloretine, phlorglucine, phloretin prepared by hydrolysis, phlorglucine mono-ethyl ether, heptacetate of phlorhizin, and trimethyl phlorhizin. He concluded that glucose such as is present in phlorhizin is dispensable because phloretin itself is relatively active. On the other hand trimethyl phlorhizin is totally inactive in spite of the presence of glucose. He thought that the esterification of the phenolic (OH) group may be the cause of inactivity (heptacetate of phlorhizin becomes active on saponification, while trimethyl phlorhizin becomes completely inactive). Independent of these facts, Lambrechts observed a glucosuria of sufficient importance (40 grams per liter) without polyuria. The final conclusions are that glucose is not indispensable in the structure of the phlorhizin derivative producing a glucosuria. The OH groups are very important for their blockage suppresses activity. Glucosuria without polyuria is observed.

Bach (6) however, observed that phloretin, unlike phlorhizin, partly inhibits both oxygen uptake and carbohydrate synthesis in liver slices if present in a concentration higher than 0.001M. But when glucose is added simultaneously, phloretin showed an effect similar to that of phlorhizin on carbohydrate synthesis. This indicates that the effect of phlorhizin is probably due to the complete glucoside rather than to its constituents.

*Influence of drugs and poisons on phlorhizin activity*. If one gives phosphorus

to a fasting animal, the metabolism is disturbed in that the total nitrogen and ammonia of the urine increase, lactic and amino acids accumulate in the organism and are excreted in the urine (90)

Ray, McDermott and Lusk (120) found that phosphorus poisoning, in dogs made glucosuric with phlorhizin, does not materially change the protein metabolism or the sugar excretion. The following explanation is offered. In phlorhizin glucosuria the high protein decomposition is due to the non-combustion of the carbohydrate radicle of the protein molecule. In phosphorus poisoning the high protein decomposition is due to the conversion of this carbohydrate radicle into leucine, tyrosine and fat (fatty degeneration). Wherever the sugar from protein is not burned, there are found pathologically hungry cells, which attract fat to themselves in larger quantities than can be utilized (fatty infiltration). Infiltration takes place in the tissues in phlorhizin glucosuria. Both infiltration and degeneration take place in phosphorus poisoning, principally in the liver.

Lusk (90) found that pilocarpine increased neither the sugar nor the phosphate secretion in phlorhizin glucosuria. In this respect the secretory cells of the kidney were thought to be dissimilar from other secretory cells.

According to Anderson and Anderson (2, 3), atropine and ergotamine are found to have no definite effect on the D/N ratio in fully phlorhizinized rats on a protein-fat diet. Nor is there any evidence obtained of any action of pituitrin on phlorhizin glucosuria in rats. Ergotoxin was found to cause a rise in the D/N ratio and the percentage of sugar in the urine of completely phlorhizinized rats on a protein-fat diet.

*Clinical considerations of phlorhizin glucosuria.* Much work has been done to try to establish a "phlorhizin test" as a reliable indicator of kidney function. Work on dogs (page 260) showed that in far advanced kidney disease, no sugar appears in the urine after phlorhizin injection.

Delmare (as reported by Lusk, 90) published a monograph on his studies on human beings. He established the reaction to phlorhizin in normal and abnormal cases and noted alteration in the degree and rapidity of glucosuria in the ones with disturbed kidneys. Casper and Richter (16) separated the urine of the kidneys by cystoscopy. Normal kidneys secrete sugar at the same rate after phlorhizin injection, but a pathological kidney showed a marked decrease in sugar excretion over its healthy sister organ. In cases of neoplasms, pyonephrosis and nephritis, the sugar excretion becomes minimal or disappears altogether. They were convinced that the test was of much value but Lusk (90) concludes, after study of all the evidence, that "it is well to assume that the phlorhizin test in kidney insufficiency possesses little diagnostic value."

Benedict and Lewis (9) attempted to make clinical application of phlorhizin to the treatment of malignant tumors. They reported retrogression of rat tumors after phlorhizinization, but Wood and McLean (164) were quick to criticize this report as the "Buffalo rat sarcoma" undergoes spontaneous absorption in 40 per cent of the cases.

Schaller (133) used phlorhizin in an attempt to show that there is no ante-

partum urine secretion by the fetus. The fetal kidney is affected by phlorhizin as in newborns he found traces of sugar in the urine if the glucoside was given within 48 hours of delivery. He reported finding no sugar in the amniotic fluid and concluded that the fetal kidney excretes no urine.

Hamnitzer and Joseph (Nash, 110) claim that 2.5 mgm of phlorhizin injected subcutaneously regularly produces in pregnant women a significant glucosuria in one-half hour. In 80 control experiments, all but 7 women reacted negatively. This pregnancy test has not been accepted.

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# THE PHYSIOLOGY OF THE PROSTATE GLAND

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From the research standpoint, the prostate gland is perhaps of greatest interest for 3 reasons its dependence on hormones, the remarkable nature of its secretion, and the frequency of neoplastic growths in the gland in several species including our own

*The female prostate* Para-urethral glands homologous to the male prostate and with identical histological appearance occur in certain female creatures In women Virchow (139) observed small nodules in the proximal portion of the urethra, which sometimes contained concretions similar to the prostatic calculi of men Most of the observations concerning the para urethral glands and the effects of hormones upon them have been made in the rat The glands are located caudal to the bladder in a position similar to the ventral prostate of the male, they are pale pink in color, contain a watery secretion and the weight may reach 70 mgm (47) The incidence of prostate glands in females is affected by genetic influences, in some strains of rats (92) the incidence was 93 per cent while in other strains no vestiges or rudiments were discernible In the gross the prostate of females is visible normally only in early life and in the last third of pregnancy, as well as in the first 2 weeks of subsequent lactation (10), exception—in the multimammate mouse *Mastomys*, a large prostate is present in all normal females (5) and it hypertrophies during the luteal phase of estrus In 1 strain (92) primordia of the prostate were first observed in both males and females at 19½ days of fetal life, 10 days after delivery the ducts and lobules showed lumen formation and on the 25th day most of the acinar cells showed evidence of secretion, while between age 30 and 40 days evidence of involution occurred (92, 115), the gland persisted for the whole lifetime of the rat but continued to show this involuted appearance (80)

The female prostate is stimulated by androgens and depressed by estrogens and castration Korenchevsky and Dennison (80) found a greater incidence of prostates with larger size in females injected with androgen than in uninjected controls and testosterone caused the formation of the prostate in ovariectomized rats (19, 78), dorsolateral prostates and coagulating glands can be induced in females with sufficiently large dosages of this hormone (93) In a series (47) of normal untreated virgin rats the incidence of prostate was 9.4 per cent in rats injected with estrone 0 per cent, and in rats injected with testosterone propionate 53.3 per cent The glands are atrophic in spayed females (80) Price (117) transplanted female prostatic tissue into other young and old, castrate and intact rats, the development was mostly good in normal males and castrate females and was poor or absent in castrate males and normal females

The rest of this paper is concerned with the prostate gland of the male

*Essentiality of the prostate* Clearly the prostate is unessential to mammalian life since in some females it is absent It is also unessential for fertility, Young



(150) was able artificially to inseminate guinea pigs with sperm from the isolated epididymis, with resulting pregnancy. Excision of the prostate and seminal vesicles abolishes fertility (131). No doubt the prostate is essential for fertilization by the natural method in mammals since the amount of fluid in which the sperms are contained in the vas deferens is so small that, without dilution, it could hardly be delivered from the urethra at ejaculation, thinning and increasing the volume of the sperm, then, are functions of the prostate.

*The prostate is not an endocrine gland.* It has been claimed that the prostate gland elaborates an internal secretion, but the evidence is oblique and unconvincing, the arguments are based on 3 types of experiments. It was stated (128) that prostatectomy in dogs was followed by cessation of spermatogenesis and that the feeding or injection of glycerin extracts of the prostate prevented the atrophy, however the caging of dogs itself (64) is often followed by atrophy of germinal cells which recover spontaneously, the housing and diet remaining unchanged.

Extracts of the prostate of the bull, both lipid and watery, injected into rabbits for many days (77, 81) sometimes produced a negative nitrogen balance, the increase of nitrogen excretion was greater when simultaneous extracts of prostate and testis were injected. The results were inconstant. It was suggested (77) that the prostate has an internal secretion affecting nitrogen metabolism and that it is in a synergetic relation with the testis.

Macht (87) found that desiccated prostate fed to tadpoles hastened somewhat their metamorphosis into frogs, although not so rapidly as feeding thyroid substance, in addition to these slight effects there occurs greater shortening and differentiation of the alimentary canal (53) than in the control tadpoles. The effects of prostatic substance seem not to be specific (118) in promoting metamorphosis since extracts of many organs had comparable effects. Prostate feeding (89) had no effect on behaviour or ability to solve maze problems in rats.

*Investigative techniques.* The principal methods of investigation of prostatic function depend on determination of size of the gland, its cytology and quantitative and qualitative studies of its secretion. The cytological evidence of secretion consists of light areas in the lumen region of the cell which appear in the rat at age 12 days (114) and disappear in the adult within 4 days after castration (101), clearly this morphologic type of evidence is largely qualitative and inferential.

Prostatic transplants grow well in the anterior chamber of the eye (51, 83, 107) and variations in size have been recorded by photography. The grafts decrease in size after orchietomy and increase when androgen (104) or gonadotrophic hormones are injected (83, 104), continued injections of a single type of gonadotrophin (e.g., chorionic) however do not lead to long continued progressive increase of size (83, 104), apparently because of immunity to the injected protein since later administration of another type of gonadotrophin, such as pregnant mare serum, causes an additional response.

Study of the secretion has provided quantitative data indicating more delicate changes in the prostate than the foregoing methods. The secretion may

be studied directly or by indirect methods. Indirect methods concern measurement of acid phosphatase in the blood or urine, acid phosphatase values in serum are increased in certain patients with metastatic cancer of the prostate (45) and serial study of this enzyme in clinical patients gave the first indication of the activity of hormones on disseminated human prostatic cancer (62). The urine of normal adult men (125) contains 3- to 5 fold greater amounts of acid phosphatase than that of women or children and, remarkably the daily amount of enzyme excreted in a single individual is nearly constant.

Direct studies of prostatic fluid have been made principally in man by massage of the prostate and collection of the secretion from the urethral meatus. Surgical procedures have been used in the dog and the boar (96).

Eckhard (24) first prepared a fistula of the prostate in the dog and discovered that stimulation of the *nervus erigens* caused abundant prostatic secretion, the dog has a well developed prostate but no seminal vesicles. Farrell (31) devised an operative method in 2 stages for the dog whereby many acute observations were made. At the first operation the bladder is separated from the prostate and an anastomosis is made between the bladder and penile sheath, followed later by establishing a urethral fistula in the perineum. Difficulties in the collection of fluid made chronic observations impossible in this otherwise ingenious technique. The first quantitative collections of prostatic fluid in the dog for long periods involved a new technique (64) whereby the urine was deviated through a metallic cannula after isolation of the prostate from the bladder and a ventral slitting of the prepuce. The dogs survive for long periods in good condition and collection of prostatic fluid is easy and complete.

*The secretion of prostatic fluid.* The prostate excretes small amounts of fluid at frequent intervals (resting secretion) and the secretion is greatly augmented by parasympathetic stimulants (stimulated secretion). Resting secretion is discharged usually from the urethra in the urine. Judging from the daily amount of acid phosphatase excreted in the urine (125), average 613 units per day, the amount of prostatic fluid secreted by man is in the order of 0.5 to 2 cc. per day. In the dog resting secretion is about 0.1 to 2 cc. per hour (3, 34) after the urine has been deviated by the prostatic isolation operation (64) the 'tree reflex' is well preserved and dogs approach the legs of laboratory tables and void prostatic fluid at frequent intervals.

Prostatic secretion is primarily a function of androgenic activity, and secondarily of chemical and nervous stimulation. Rises of acid phosphatase in the urine occur after sexual stimulation (125) in man. In dogs frequently a steady output occurs for months while less commonly a plateau of secretory activity is interrupted by high peaks (64). Normal dogs may secrete as much as 4 times the weight of the prostate in 1 hour, but very large glands in spontaneous cystic hyperplasia do not give correspondingly large amounts of secretion, although the cysts communicate with the ducts, for example, a dog (60) whose hypertrophied prostate weighed 102 grams secreted from 7 to 11 cc., whereas a young normal adult of the same body weight secreted 24 to 38 cc., the prostate weighing 10.8 grams.

Pilocarpine hydrochloride, 6 mgm. intramuscularly, provides a good output

of secretion in normal dogs, usually without accompanying emesis and about 95 per cent of the secretion occurs in the first hour after injection. Increasing the amount of pilocarpine increases the secretion (64) but not in a simple linear relationship and the gland does not become refractory to pilocarpine, although some signs of "prostatic fatigue" are seen after repeated stimulations in a very short time.

It was claimed (33) that occlusion of the outflow of prostatic secretion caused atrophy of the gland but the experimental technique was defective, plugging the ducts with a transplant of muscle (61) did not lead to prostatic atrophy in dogs injected with androgen.

Starvation of dogs injected with androgen for 3 weeks led to an increased secretion of prostatic fluid and definite growth of the prostate, Pazos (109) observed further that the composition of the secretion was not disturbed by inanition.

*Effect of nerve stimulation and drugs on prostatic secretion.* There is no secretion from immature (99) or castrate dogs (64). The secretory pressure of the adult canine prostate has been found to be 16 to 18 (99), and 17 to 26 (34) mm of mercury respectively. Stimulation of the hypogastric nerves (34, 99, 127) and the *nervi erigentes* (24, 99, 148) induces prostatic secretion. Faradic stimulation of the hypogastric nerves produced either large (99, 126) or small secretion (148) in the dog. In man stimulation of this nerve causes ejaculation of semen (6).

Parasympathetic stimulating drugs greatly augment prostatic secretion. Epinephrin, nicotine and acetyl choline (34) caused a slight increase of secretion and pilocarpine a great increase (34, 64, 99, 148), atropine antagonizes pilocarpine stimulation and results in cessation of secretion (34, 148).

Strips of the normal prostate gland exhibit no rhythmic contractions (88, 94, 95, 140) but these occur in the prostate of castrate rats (94, 95) and the administration of androgen to the castrate reduced the spontaneous contractions. Epinephrin and barium chloride (88, 140) produced an increase of tone in prostatic strips while in most species pilocarpine added to the testing bath was without effect, in the rabbit however pilocarpine (140) produced increases of tone and rhythmic activity not detected in other animals except in the prostate of castrate rats (94, 95).

*Pharmacodynamic effects of prostatic secretion.* The intravenous injection of semen is always followed by a rapid fall of blood pressure (38). Prostatic fluid and extracts of the prostate gland have powerful biodynamic actions. Prostatic extracts of the dog and bull when injected in the rabbit cause a pressor effect (4, 135) followed by a marked lowering of blood pressure (26, 28), intravascular clotting and death (16, 73, 135). The pressor effect is probably due to epinephrin since this substance has been demonstrated in the prostate in considerable amounts (18, 27). Euler has studied the vaso-depressor substance, *prostaglandin*, occurring in extracts of the prostate and in prostatic fluid obtained at autopsy from man, the substance has acid properties, is dialyzable and soluble in water and alcohol, it lowers the blood pressure and increases intestinal motility in the atropinized rabbit and dilates the vessels of the hind limbs of the frog (26,

28, 29), larger quantities were found in the prostate of the adult than in children (28). Another highly active substance, *vesiglandin*, was found in the prostate of the monkey (28), thus lowered the blood pressure but had no, or only a weak effect on isolated intestine. These agents which produce vasodilatation and strong contraction of smooth muscle occur in the prostate and seminal vesicle in larger amounts than in many other organs and are assumed by Euler (28) to function in the emptying of these glands. The prostate of the sheep contains the same amounts of vasodepressor and smooth muscle relaxing substance as human

TABLE 1  
*Chemical composition of human and canine prostatic fluids*

	MAN RESTING FLUID	DOG PILOCARPINE STIMULATION
pH	6.3-6.45 (67)	5.29-6.16 (64)
Values per liter of fluid		
Water gram	927-936 (67)	981 $\pm$ 3 (64)
Sodium meq	149-158 (67)	159 $\pm$ 2.6 (64)
Potassium, meq	28.7-31.4 (67)	5.1 $\pm$ 0.2 (64)
Calcium meq	28.7-32.7 (67)	0.3 (64)
Chloride meq	34.8-46.1 (63)	160 $\pm$ 2.7 (64)
Acid-soluble P meq	0.65-1.77 (63)	Trace (64)
Carbon dioxide mM	3.1-5.4 (67)	0.8-0.9 (64)
Values per 100 cc of fluid		
Total nitrogen, mgm	295-511 (67)	154 (64)
Non-protein nitrogen, mgm	30-90 (67)	22 (64)
Total protein gram	2.46-2.64 (67)	0.8 (64)
Glucose mgm	Trace-16.4 (63)	0-30 (64)
Ascorbic acid mgm	0.54 (3)	0.76 (3)
Citric acid grams	0.48-2.68 (66), 7.0 (123)	0.0026 (66)
Acid phosphatase King and Armstrong units (76)	255-1727	3-286 (3)
Alkaline phosphatase King and Armstrong units (76)		0-106 (3)
Total lipids mgm.	286 (124)	
Cholesterol mgm.	62-105 (124)	
	86-618	130-210 (106)
Specific gravity	1.022 (106)	1.005-1.008 (3)

prostate, the content of organs such as liver, testis and muscle was less than 1 per cent of the prostate (30). The lowered blood pressure following intravenous injection is abolished by exclusion of the liver from the circulation (29).

**THE COMPOSITION OF PROSTATIC SECRETION** The chemical composition of prostatic fluid (table 1) is remarkable from the standpoints of inorganic, organic and enzyme chemistry. All of the data for human prostatic fluid relate to resting secretion obtained following prostatic massage, amounts of fluid obtained are usually less than 1 cc so that complete analyses cannot be made on a single sample and it occasionally is contaminated with fluid from the seminal vesicle or ejaculatory ducts. Quantitative data for dog prostatic fluid are more exact since

technical difficulties are less Compared with stimulated secretion, the resting secretion of dogs (3) is more turbid, more alkaline and contains less chloride, observed values, pH 6.6, chloride 104 m eq per liter

*Inorganic* The prostatic secretion is more acid than plasma Stimulated dog prostatic fluid approximates 0.16 normal NaCl (64) Human prostatic fluid contains considerably larger amounts of sodium, potassium and calcium (67) than plasma, on the other hand there are deficient anions with low levels of chloride, bicarbonate and phosphate Other anions, namely, the amino acids (20 mgm per cent), proteins—peptones and proteoses, and citrate balance the cations inadequately for the observed pH of 6.45

*Organic* Most of the proteins of human prostatic fluid are derived proteins which are not coagulated by heat and pass readily through semi-permeable membranes—proteoses (39, 67, 111, 119) The content of glucose in prostatic fluid is exceedingly low (63) Scherstén (123) and others (21, 66) found that human prostatic fluid is rich in citrate No doubt citric acid is a factor in the accumulation of calcium, since other fluids rich in calcium salts (e g, milk and urine) are rich in citrate, and citrate also functions in preventing the precipitation of calcium salts, however precipitations of calcium phosphate (apatite) and cholesterol in the form of calculi (59) often occur Even in young men such apatite deposition occurs in the "corpora amylacea" The spermine content of human prostate is remarkably high (49) (0.1 gram per 100 grams of fresh tissue) and probably most of the spermine of semen is derived from this organ (48), this base does not activate spermatozoa

*Enzymes a Phosphatase* Kutscher and Wolbergs (85) discovered that acid phosphatase is present in prostate gland and prostatic fluid in very large amount This enzyme is a chemical secondary sex characteristic, Gutman and Gutman (42) observed small amounts in infancy increasing during puberty to high values in the adult and that traces in the prostate of immature monkeys (43) became greatly augmented by androgen administration No function for this remarkable enzyme has been discovered

*b Fibrinolysin* Proteolytic enzymes were discovered in prostatic fluid (66) in a study of liquefaction of human semen when it was found that these fluids rapidly liquefied clotted blood and fibrin Human semen contains fibrinolysin derived in large amount from the prostate gland This enzyme, a physiological constituent of the prostate of man, resembles closely streptococcal fibrinolysin (137) previously recognized only in association with bacteria Human blood is lysed well by this enzyme, which lyses rabbit blood poorly and beef blood not at all Contrasted with trypsin, human prostatic fluid does not act on denatured hemoglobin (66) and digests fibrinogen with difficulty, contrasted with streptococcal fibrinolysin, the blood of men resistant to this enzyme is easily lysed by prostatic fibrinolysin—an immunologic difference, both streptococcal (97) and prostatic (98) fibrinolysins are inhibited by crystalline trypsin inhibitor

*c Fibrinogenase* It was observed that the prostatic fluid of the dog destroyed fibrinogen more readily than fibrin, the activity being attributed to fibrinogenase (66) Both fibrinolysin and fibrinogenase are chemical activities as yet unidenti-

fied as chemical entities. Both human and dog prostatic fluids contain these activities but in different concentration. Human semen contains much fibrinolysin, little fibrinogenase, dog semen contains little fibrinolysin, much fibrinogenase. The fibrinogen of man in certain disease states is not destroyed by dog fibrinogenase (66). These agents may be maintained for years in refrigerated prostatic fluid.

d *Miscellaneous*. The prostatic secretion of normal dogs (70) clots oxalated or heparinized rabbit and beef plasmas (these fluids are resistant to the activity of fibrinogenase) but does not clot fibrinogen alone, human prostatic fluid does not coagulate oxalated plasma. The tryptic activity of dog prostatic fluid ranges from 0.1 to 1.1 units. Thromboplastin (66) is present in prostatic fluid as in the juice of many other tissues.

The prostate gland and fluid of the dog have been reported to contain amylase (75). We have confirmed this observation but found it to be inconstant. A germicidal power (149) in dog prostatic fluid has been described against *B. coli*, *S. aureus* and streptococci, neither complement nor lysozyme was demonstrated. Camus and Gley (14, 15) found that the prostatic fluid of the guinea pig contains an enzyme, *vesiculase*, which coagulates the seminal vesicle secretion, it is unable to clot blood or milk. Diamine oxidase is present in considerable quantity but is less concentrated (151) in prostatic tissue than in semen.

*Drugs*. Alcohol (32) is excreted in dog prostatic fluid (90–594 mgm. per cent) after ethyl alcohol administration. Sulfonamides (35, 58, 84) likewise are excreted.

*Lipids of the prostate*. Thompson (136) described the laminated corpora amylacea normally found in the prostate of adult man and monkey (and only in these species) and described yellowish refractile bodies in prostatic fluid. Fürbringer (36) discovered that the opalescence of prostatic fluid is due to fat droplets which he named "lecithin granules"; this lipid displays double refraction in polarized light (112, 113). Lipids are easily demonstrated in the epithelial cells (110, 147) and their presence in prostatic fluid represents a true secretion. The cholesterol content (133) of normal prostatic tissue (0.8 gram per cent) is lower than that of prostatic adenomas (1.10 grams).

Scott (124) found an average total lipid content of human prostatic fluid of 286 mgm. per cent, approximately half the concentration found in human plasma. Total phospholipids constituted over 60 per cent of the total lipid value. The moist-ether soluble phospholipid consisted of cephalin. Lecithin was not demonstrable indicating that the term "lecithin bodies" is not applicable to the fat bodies found in prostatic fluid. His values for total cholesterol in prostatic fluid ranged from 62 to 105 mgm. per cent considerably lower than the range of 86 to 618 mgm. per cent reported by R. A. Moore (106). These analyses indicate that there is little if any neutral fat in prostatic fluid, the phospholipids and cholesterol accounting for almost all the total lipid found. Microscopic examination of prostatic fluid from normal men reveals lipids in gross aggregates while the lipids of plasma are of colloidal particulate size or are in solution. In certain prostatic inflammatory states much or all of the lipid is found in macrophages (colostrum corpuscles) in the prostatic secretion.

*Effect of prostatic fluid on spermatozoa* The addition of prostatic fluid to sperm is said to increase their motility, the experiments have not been carefully carried out and it is impossible to exclude non-specific factors, such as dilution of thick sperm, the effects of inorganic ions and changes in gas tension as the cause of the acceleration. Saline admixed with prostatic fluid increased sperm motility and preserved vitality longer than occurred in the saline (54, 127, 131, 148) solution used, sodium chloride, 0.7 per cent, which however itself is toxic to spermatozoa (54). The addition of dog prostatic fluid to testicular spermatozoa (which are commonly motionless or nearly so) produced active motion (143), the same result was achieved with saline.

Ivanow (71, 72) reported that prostatic fluid increased the motility of sperm temporarily but did not represent the most favorable media for life of the sperm, utilization of oxygen was less rapid in prostatic fluid than in boiled prostatic secretion or saline.

TABLE 2

*Chemical composition of fractions of the ejaculate*

Glucose arises chiefly from seminal vesicle, the other components are excreted chiefly or exclusively by the prostate.

COMPONENT	FRACTION OF EJACULATE	
	First	Last
Citrate, mgm per cent (123)	770	120
Calcium, mgm per cent (123)	52	20.4
Acid phosphatase units, 1 cc (44)	2760	300
Glucose, mgm per cent (91)	157	377
Fibrinolysin, minutes per test (65)	120	288

*Time of emptying of the prostate during ejaculation* In man it is known that the prostate discharges most of its secretion before the seminal vesicle in, roughly, a one-two relationship during ejaculation. The evidence is chemical in nature obtained on ejaculates fractionated according to time of delivery from the urethra (table 2).

*Metabolism of isolated prostatic tissue* The oxygen consumption of the prostate of the normal dog ( $QO_2$  4.3) and rabbit ( $QO_2$  6.2) is approximately half that of kidney. Aerobic glycolysis ( $Q_L^{O_2}$  0.97) is high indicating a deficient oxidation of carbohydrate in the normal (2). The carbohydrate utilization of human prostatic adenoma is characterized by a low respiration, low pyruvate utilization, and high glycolysis, anaerobic and aerobic.

THE RELATIONSHIP OF ENDOCRINES TO THE PROSTATIC FUNCTION In the normal prostate the acinar epithelium is composed of tall columnar cells, 16–28  $\mu$  in the guinea pig (121), 20–30  $\mu$  in the rat (114), 14.4–27.5  $\mu$  in man (69). Secretion is only possible from this tall epithelium which in turn is a function of androgen, and when androgen is withdrawn sharp decreases in height occur (69, 101).

When orchiectomy is done in dogs, a measurable decrease of secretion is observed within 24 hours and prostatic secretion ceases 7 to 23 days after castration (60, 64). Usually orchiectomy is followed by prompt cellular regression of the prostate, changes in the cytology of the rat being observed promptly (101). There is loss of the light areas within 4 days and then progressive reduction in epithelial height, nuclear size and acinous diameter, the epithelium becoming pseudostratified and the nuclei pycnotic (116). Exceptions to this rapid involution after castration were noted in benign hypertrophy in man where decreases in height of the epithelial cells were not marked at 29 days (69) but were severe at 93 days. Also, in the guinea pig (37, 121, 122) involutionary changes develop slowly after castration. Development and maintenance of well differentiated epithelium occur in the prostate of rats and mice castrated at 1 to 3 weeks of age—the Price effect (114) and is apparently associated with the secretion of androgen by the  $\lambda$  zone in the adrenal cortex (55, 56) since adrenalectomy (9, 57) reduces the prostatic weight, the anomaly disappears spontaneously about day 40.

Testosterone propionate, 10 mgm daily in sesame oil, gives measurable prostatic secretion in immature dogs after 3 to 7 injections (64), and there is a steadily rising secretory curve for at least 5 months, in hypogonadism in the male androgens initiate and increase prostatic secretion but when administered to normal man testosterone had little effect on the seminal output (52). There is further evidence that androgen is less effective in intact males than in castrates, the daily administration of 0.1 to 0.5 mgm of testosterone propionate per kilogram to rabbits with intraocular prostatic transplants (82) produced an increase of area of the graft for 2 weeks followed by a stillstand or regression, the same treatment in castrate rabbits produced a continuous increase for at least 3 months.

Burkhart (7, 8) studied the effects of androgen on the prostate by the colchicine technique, the male hormone effects an increase of mass of the prostatic epithelial cell followed by cell division. A single dose of testosterone propionate, 0.1 mgm, in a castrate rat weighing about 170 grams causes an increase of cell volume of the epithelium of the ventral prostate detectable 23 hours after injection followed by mitotic division at 35 hours, the effects of androgen are evident for 2½ days after this single injection when the cells revert to a castrate condition.

Soon after the introduction of crystalline androgens it was found that the characteristic cytologic effects of androgen on the prostatic epithelium could be abolished under certain conditions by simultaneous administration of estrogenic hormones (50, 74, 152, 153, 154) in the mouse, rat and monkey. Also with respect to the prostatic secretion estrogen is able to nullify the effect of androgen (60), the dosage of estrogen has been determined whereby the rising curve of prostatic secretion induced by injected androgen is converted to a plateau (neutralization), and larger doses completely vitiate the secretory effects of an androgen. By this type of biological "titration" (60) it was found that daily injections of diethyl stilbestrol, 0.4 mgm, neutralized testosterone propionate, 10 mgm, similarly administered (a ratio of 1:25) and larger doses of this estrogen



caused a cessation of secretion. When estrogen is administered to a castrate or immature dog, the acinar elements remain uninvolved while in the ducts, the lining of the posterior urethra and the utriculus, columnar epithelium is replaced by stratified squamous cells, when the acini are lumined (action of androgen) this epithelium also becomes squamous and stratified (60). This metaplasia is reversible by androgenic treatment (120). Involvement of the utriculus with squamous cells is the first cytologic sign in the dog of estrogen dominance.

The prostate gland in late fetal life and in new born boys usually is the site of epithelial metaplasia in the utriculus and the collecting ducts (1), this was attributed for theoretical reasons (46) to penetration of hormones from the mother through the placenta. The metaplasia is due to estrogen. Burrows (11) produced these changes by injecting estrogen in adult mice and restitution to the normal state followed a cessation of estrin, estrogen administered to infants (129) maintained these changes for 7 months—an age when the prostate in normal babes would have resumed the customary inactive appearance.

In the intact mouse (12, 86) dog and monkey (141) administration of estrogen for long periods leads to urinary retention with uremia and death of the animal, the posterior lobe of the prostate and the utriculus become enormously distended with retained secretion. In the rat (79) and monkey (153) male hormone administered with estrone completely prevented the pathological changes in the prostate. Since the introduction of anti-androgenic methods of treatment of cancer of the prostate by Huggins and Hodges (62) considerable numbers of men have been treated with large doses of estrogen for prolonged periods without "estrogenic urinary retention", man is, apparently, the only mammal in which this phenomenon does not occur. Estrogen (138) increases growth of muscle and connective tissue of the prostate.

Sharp decreases in prostatic secretion in the dog follow within 1 day after injection of an adequate amount of estrogen which, when maintained, leads to cessation of secretion in about 10 days (60), desoxycorticosterone acetate (68) causes only a slight secretory depression. The responses of the prostate to both androgenic and estrogenic stimulation appear to be unaffected by section of the hypogastric and sacral nerves (133).

*Androgen threshold of the prostate.* The prostate has a lower threshold to androgens than the seminal vesicle. Working with crude extracts Moore and Gallagher (100) found that about 3 times as much androgen was required to show effects in the seminal vesicle as in the prostate. This has been confirmed with pure androgens (8, 108). In the administration of estrogen to man (65) it was found that the amount of seminal vesicle secretion in the ejaculate decreases more rapidly than the prostatic secretion, the ejaculate becomes less solid, liquefies more quickly and the concentration of glucose decreases more rapidly than fibrinolysin (65). There is a differential reaction of the prostatic lobes in the rat to androgen withdrawal. On the tenth day after castration involutionary changes were first detected cytologically in the anterior lobe (coagulating gland) although they had been noticed on the fifth day in the other prostatic lobes (101).

*Two functionally different prostates* For many years it had been known that coagulation resulted when prostatic fluid of the rat was mixed with the secretion of the seminal vesicle, Walker (145) observed that only secretion from a specific region of the prostate, the anterior lobes (coagulating gland) could induce clotting, the middle and posterior lobes being inactive. The coagulating gland has a slightly different cytologic appearance from the other lobes although it has the same embryologic origin (25). This gland occurs in rat, guinea pig (145) and monkey (142), cross-coagulation is possible between the seminal vesicle of one species and the anterior prostate of another (142, 144) and the fluids do not induce coagulation of blood. Citrate and oxalate do not inhibit seminal clotting. The coagulation is due to the enzyme, vesiculase (14, 15). A coagulating gland has not been detected as yet in man.

*Benign prostatic hypertrophy* This condition occurs spontaneously with frequency only in dog and man, but the disease differs in these species. Common to both is the requisite of senility. Nearly all ancient dogs (objective evidence—worn teeth and cataracts) with functioning testis (60) have this disturbance. In man it is characteristically a disease of men over 40 years of age (134), in a series of over 700 prostates, R. A. Moore (103) found the youngest man with a true spheroidal nodule in the prostate was 39 years of age and that 75 per cent of white men between 80 and 90 had histologically demonstrable prostatic nodules (102). The disease has not been experimentally produced.

In dogs the condition consists of cystic hyperplasia (40, 41, 130) of the entire prostate, does not produce urinary obstruction and recedes massively after castration and estrogen (60), following atrophy, androgen reconstitutes the disease in the cystic condition rather than in a normal state (60).

In man, in our experience, benign prostatic hypertrophy always involves only a part of the prostate, the periurethral region, in which location spheroidal nodules are invariably found in this disease, the posterior lobe of the human prostate is never involved by this adenomatous hypertrophy so that it is useful to think of this as a separate and unique part of the gland but LeDuc (23) has denied its existence as an anatomical entity. Also the prostatic epithelium is always of tall columnar type (69)—evidence of the presence of functioning androgens. The disease is rare among Chinese (17).

Differences of opinion exist as to the effect of castration on human prostatic hypertrophy, Deming (20) and others (103) were unable to find evidence that castration brought about atrophy of the hyperplastic tissue while White (146) and many other urological surgeons (13) observed great decrease in the size of the hypertrophied prostate. We (69) observed decreases in prostatic size and also in the height of the epithelium 3 months after castration in 2 men, however enucleable spheroids of muscle, connective tissue and atrophic epithelium remained. In men with prostatic hypertrophy, the urinary excretion of androgen and estrogen (22, 105) is decreased with respect to normal young men.

In sum, human prostatic hypertrophy is an involvement of the periurethral portion of the prostate by nodular growths occurring commonly in Caucasians rarely in Chinese. The epithelium is invariably tall columnar, so that andro-

gens must be present in physiologically effective amounts, yet much evidence suggests that androgens are not produced in as large quantity in old age as in young men, despite the fact the prostate grows. Certainly prostatic hypertrophy is a neoplastic condition. There is no evidence that estrogen has a causative influence on prostatic hypertrophy. To best account for the facts, we suggest that a decreased threshold to androgen in the spheroidal nodules permits prostatic growth in the face of decreasing androgen production.

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# CEREBRAL CONCUSSION

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The application of the word concussion to the commonly observed transient physiological effects of trauma is derived from the nature of the usual kind of causal injury, which is such as to give "shock of impact"<sup>1</sup> Such transient traumatic disorders are most obvious and most commonly observed in relation to nervous structure, and this review is particularly concerned with concussion of the brain In this connection some further definition is necessary for there are several possible transient and unrelated consequences of head injury The most obvious change in concussion is a disturbance of consciousness both immediate, consecutive, and remote, and the most satisfactory definition would appear to be that of Trotter (97) "an essentially transient state due to head injury which is of instantaneous onset, manifests widespread symptoms of purely paralytic kind, does not as such comprise any evidence of structural cerebral injury, and is always followed by amnesia for the actual moment of the accident" Thus is cerebral concussion differentiated from an injury of such levity that the subject is momentarily only dazed, from cerebral contusions which do not immediately interrupt consciousness, from certain delayed disturbances of consciousness related to syncope (Denny-Brown, 14) or resulting from increased intracranial pressure (epi- or subdural hematoma), and from disturbances of consciousness of purely psychological kind

The practical importance of the subject is twofold First there is lack of clear understanding of common sequelae to head injury, namely liability to headaches and dizziness, inability to concentrate, nervousness or unwarranted fears, various degrees of change in personality (irritability, aggressiveness, euphoria), and intellectual impairment These symptoms have often loosely been called "the post-concussion syndrome" Secondly, the effects of exposure to violent explosion particularly common in war include persistent anxiety, loss of memory, and difficulty in concentration with or without headache and dizziness similar or identical with the "post-concussion syndrome," without outward sign of direct injury to the head It is therefore pertinent to inquire whether any of the phenomena of concussion can be associated with a persistent structural disorder, what is the relationship between "post-concussion syndrome" and concussion, and whether the blast of explosive produces concussion or some other related state It should be made clear that there is already a considerable volume of clinical evidence (97, 84, 85, 16, 20) to show that the "post-concussion syndrome," though most frequently seen as a result of concussion, occurs after both milder and more severe injury The extensive earlier literature was reviewed by Strauss and Stavitsky (93), Symonds (95) and Schilder (88) The whole problem is, however, still unsettled, particularly in the rela-

<sup>1</sup> Oxford English Dictionary

tionship of post-traumatic personality changes to generalized damage to the brain. Before these questions can be answered it is necessary to inquire closely into the physiological disturbance underlying cerebral concussion.

6. *Human concussion* Careful documentation of the events immediately following the blow to the head in man is rare, for it seldom happens that a careful record is kept of the state of respiration, pulse, blood pressure reflexes, etc., in the earliest stage. The classical case is one of which a description is given by Pols (80). The following is a brief summary.

The patient fell 10 to 12 feet on his head on pavement and was seen by Pols in 4 minutes. He was completely insensible with face of cadaveric pallor, pupils large and fixed and no sign of muscular activity. Respiration was extremely slow (4-5 a minute) with unequal pauses. The pulse was full but slow (48) and very irregular. The irregularity in pulse and respiration lessened gradually after 20 minutes when vascular tension diminished. Corneal and other reflex sensitivity reappeared after half an hour. The pulse was then 60, and respirations 12-14 a minute. Consciousness was recovered after 15 hours. There was some evidence of slight cerebral contusion, for a right sided convulsive attack with some fever occurred on the fourth day.

A case of brief concussion (5 min.) followed by extensive loss of memory of events preceding the injury is related by Gussenbauer (41) but no other details of the clinical state are given. An erroneous account of Gussenbauer's case, in which details of the case cited by Pols are interposed, is given by Kocher (58) and repeated by Miller (68).

Hutchinson (54), Kramer (60), Kocher (59), Bergmann (3, 4) and other surgeons confirm the initial areflexia without however giving case details. Similar changes are commonly noted after severe head injury, where the presence of skull fracture, cerebral contusion or laceration add uncertainty to the cranial pathology, but Pols' case may be taken as a classical pattern of the immediate disturbance in man.

The prominence of disorder of consciousness in the clinical definition of concussion has been a difficulty in the interpretation of animal experimentation. This disorder in human concussion is complex enough and deserves some detailed mention here. It is maximal in degree immediately after the injury and thereafter progressively lessens. If the disturbance is severe, an initial stage of coma ("absence of any psychologically understandable response to external stimuli or inner need," Brain Injuries Committee (6, 1941)), is usual. Within a few seconds or minutes (possibly as long as a few hours, *vide infra*) simple maldirected response to painful stimuli or loud demands, appear. This state is commonly called *semi-coma*. This is in time followed by a phase of automatic behavior, called mental "confusion," in which responses of increasing appropriateness are made, but where there is disorientation in time, place, and person. There is subsequently no memory of any of these states (post-traumatic amnesia). The improvement in the stage of confusion is usually so rapid that accurate observation of the changes are difficult. Occasionally the whole disorder is spread over many days, or even weeks, in cases without sign of complicating injury, and if as some contend (Symonds, 94, Denny-Brown, 16) such cases may be considered as severe con-



cussion, the progress of the disorder may then be followed in greater detail. Recognition of relatives, recovery of continence of bladder, and progressive lessening of restlessness then document the slow improvement in the state of awareness. The mental confusion that accompanies this stage has clear evidence of disturbance of perception, and several authors (Schilder, 87, Symonds, 94, Paterson, 78, Paterson and Zangwill, 79) have used the principles of Gestalt psychology in relation to the restriction of the field of consciousness to explain the confused behavior. Orientation in time is usually the last process to recover (70), and is usually coincident with recovery of the patient's memory of the day before. His continuous memory also begins from that date. He may subsequently have a memory for isolated "islands" of happenings in the period of confusion, usually of some dramatic circumstance, which he is unable to relate to the remainder of his memory.

Retrograde loss of memory is a very distinctive feature of concussion. The gap is usually of only a few seconds duration, so that the patient cannot remember the actual injury. The extent of retrograde defect is not proportional to the duration of post-traumatic amnesia (86) and is often only a fraction of a second in severe cases. During the period of confusion at a stage when he can answer questions relatively intelligently (but is still disoriented) the patient is found to have a long retrograde amnesia, commonly of years, and if very confused he may be under the impression that he is reliving events in the distant past. This retrograde loss progressively shrinks and commonly continues to diminish after recovery of orientation, eventually reaching the brief interval of true retrograde amnesia. It has not proved possible to recover either the final true retrograde loss or post-traumatic loss by methods of hypnosis or dissociation as in hysterical amnesias. There is good evidence in some cases that the patient can remember the events leading up to a mild injury, but not the actual impact, immediately he recovers from the brief coma, and yet later have this same memory buried in a longer period of retrograde amnesia (7, 73).

*Experimental concussion.* There has been no attempt to date to identify various states of awareness in experimental concussion except the recent work of Dow and his associates (22) who have found that conditioned reflexes in dogs were affected by blows to the head of less intensity and for a much longer time than was reflex activity at lower levels. At the beginning, therefore, the investigator has to decide what criterion he is to use for the identification of concussion in his experiments. A state of lack of reaction comparable to coma is readily recognized in the condition in which an animal is said to be "stunned," but amnesia in the human sense has no animal counterpart. The situation is further complicated by the necessity for anesthesia in the course of experimentation. The early investigators accordingly adopted the criterion of immediate death without any lesion of the brain visible to the naked eye as indisputable evidence of the occurrence of concussion.<sup>2</sup> It is however exceedingly difficult to accomplish this and the demonstration by Koch and Filene (58) that an

<sup>2</sup> For historical reviews of the numerous hypotheses that have been advanced the reader is referred to the papers of Gussenbauer (42), Bergmann (3, 4), Polis (81), Kocher (59), Duret (24), and Denny-Brown and Russell (15).

animal could be killed by a series of light blows without hemorrhagic lesions first showed that "concussion" in this sense was possible. Polis (81) in 1894 demonstrated the immediate rise and subsequent fall of blood pressure that followed each such blow, with final failure after the last blow. He proceeded to demonstrate that a greater rise in blood pressure and cessation of respiration could follow one severe blow, and be followed by fatal respiratory and vasomotor failure without hemorrhagic lesions being necessarily present. He accordingly considered concussion as being essentially the result of traumatic paralysis of the bulbar centers, and found that a tangential impact of a bullet on the cranial vault was a convenient method of reproducing the condition. Bergmann (3) had since 1880 emphasized the great slowing of the pulse which usually but not always accompanies concussion and makes this an objective part of the definition of the condition. Polis (81) noted the vagal slowing and also made extensive investigations of the mechanism of vasomotor changes which will be referred to later. The vascular and respiratory changes were confirmed by Miller (68), who also stunned unanesthetized rabbits for one minute and on recovery injected Trypan blue intravenously, on 3 successive days, finding no staining of the brain or meninges in animals that had undoubtedly been rendered immobile by the injury 5 days before. Jacob (56) had also investigated microscopic changes after recovery from stunning in animals, finding petechial hemorrhages in some, none in others, and not adopting any standard for concussion. Miller (68) repeated a simple experiment reported in 1877 by Witkowski (107) in which it was demonstrated that a frog with excised heart, in surviving for some minutes, could in that period momentarily be stilled by a blow on the head. After a few moments it could right itself and continue activity. The experimental use of "stunning" an animal to investigate concussion, and the demonstration that this phenomenon could occur without vascular lesions, and indeed without vascular mechanism had therefore been accomplished more than a decade ago, but in 1940 this evidence was still not generally accepted.

In a monograph published in 1901 Kocher (59) and his pupils described similar respiratory cessation and vagal slowing of the heart produced by transient severe pressure on the dura, and proposed a hypothesis of the mechanism of concussion based upon a conception of transient ischemia induced by the deformation of the skull, with subsequent vascular stasis. Kramer (60) and Félizet (8) had shown that the skull is deformed by pressure and had demonstrated a rise of internal pressure when the dried human skull was compressed. Knauer and Enderlen (57) with more refined experimentation found that a blow upon the skull could indeed cause vagal slowing of the heart and a transitory swelling of the brain. Scott (90) measured the intracranial pressure under similar circumstances, allowing a weight to fall on the supported head, and found a peak pressure of 300 mm. Hg at the moment of impact of a blow sufficient to abolish any response to noxious stimuli for 2 to 10 minutes. He demonstrated that a momentary passive increase of intracranial pressure of the same degree and lasting about 5 seconds also abolished responses for 5 minutes. The case for transient anemia as explanation of concussion appeared to be complete.

The mechanism that had been demonstrated however was essentially that of

cerebral compression Denny-Brown and Russell (15) reviewed the above and related studies, and pointed out that it had not been demonstrated that a generalized increase of intracranial pressure occurred in the most usual kinds of injury when the head was thrown suddenly and violently into motion, or suddenly stopped when in motion. It also remained to be explained why very brief pressure should produce such lasting disorder of nervous function. These investigators observed the effect of a blow on the unsupported head in lightly anesthetized cats and described an orderly succession of recovery of nervous function following its immediate abolition by a certain degree of injury. They chose the recovery of the corneal reflex as a convenient standard by which to measure the duration of traumatic disturbance of cerebral function. Transitory abolition of the corneal reflex was seen to accompany total loss of reaction in an unanesthetized monkey accidentally observed. Such abolition, in the absence of any trace of hemorrhagic lesion in the brain, brain stem or cervical cord, was accepted as evidence of concussion. They stated objections to other criteria. Other reflexes could not be elicited with such regularity, although they also were abolished for a variable interval of time by a blow sufficient to efface the corneal reflex. It is open to any investigator to nominate which of these, or other changes he shall adopt as his criterion. Loss of the corneal reflex has been found to be a satisfactory index of the reaction by Gutiérrez-Mahoney (43), Groat *et al* (38), Walker *et al* (98), Clarke and Ward (12), and others. Gurdjian and Webster (40), using dogs anesthetized with morphine, have stated that their animals could be rendered briefly unconscious by head injury, as judged by lack of reaction to compression of the tail, without loss of corneal reflex, though in all moderate and severe grades of injury the corneal reflex was lost. Response to pressure applied to the tail may therefore be a more sensitive indicator of general reactivity. No doubt more sensitive tests, such as reaction to visual threat, could be used if the level of anesthesia permitted. It is an essential part of the thesis advanced by Denny-Brown and Russell (15) that the initial disturbance should be generalized and maximal, if varying in duration in various mechanisms. Just below the threshold for characteristic respiratory and vasomotor effects a brief lessening of response in corneal reflex and swallowing was found. Such heightening of threshold may well give the impression of absence of response to tail pressure or other tests. The response to tail pressure cannot in any case be accepted as indication of loss of "consciousness" as claimed by Gurdjian and Webster (40). It remains to be shown whether amnesia, which appears to be the only true index of the disturbance of consciousness, can be produced by lesser degrees of injury than will abolish reflex function. Inactivity of a conditioned stimulus without impairment of the brain stem mechanism as found by Dow and Raaf (22) is interesting in this connection, though it is necessary to be certain that the effect was not just the distraction of attention such as would be produced by any loud sound or other sensory stimulus ("external inhibition").

The question of anesthesia is manifestly of great importance. Initial vinyl ether, followed by novocaine infiltration of operative fields, as used by Walker *et al* (98), appears to be most satisfactory. Denny-Brown and Russell (15)

and Williams and Denny-Brown (103) used nembutal, controlled in other experiments by the use of ether alone, but the anesthesia with these had to be very light indeed, especially for electroencephalographic recording. Under deep anesthesia the rise in blood pressure with concussion is less marked and may be absent, the respiratory change is reduced to a simple pause, and all excitatory effects lessened or abolished. The use of morphine as anesthetic appears likely to produce equivocal reactions if a noxious stimulus is used as test indicator.

*Acceleration concussion* Denny-Brown and Russell (15) observed that it was possible to graduate the effect produced by the intensity of trauma on the unsupported head. Between an intensity of injury sufficient to kill the animal with one blow (usually with contusion of the brain and upper cervical cord, but occasionally without lesion visible to the naked eye), and an intensity which did not cause loss of corneal reflex ("subconcussive" injury), was a wide range within which abolition of the corneal reflex for variable intervals of time could be brought about. The duration of the other phenomena varied with that of the corneal reflex. By means of a graduated pendulum they were able to measure the intensity of such injuries. It was apparent that the trauma under such circumstances had to have a critical velocity as well as energy, for however great the moving mass, at slow speeds of striking the head was simply pushed gently aside. If the head is prevented from moving by some clamp or heavy obstruction, the effects produced would be more directly related to the mass of the striking object, and the ability of the skull to withstand the crushing effect of momentum.

The two modes of injury are clearly different, but whether the difference is of practical importance will be discussed further below. Denny-Brown and Russell (15) reported that in 10 patients observed by them with head injury from crushing violence only one had immediately lost consciousness with retrograde amnesia, in spite of the production of cranial fractures and cranial nerve injuries in all.

By the use of the pendulum, striking the movable head, it was found possible to abolish the corneal reflexes without hemorrhagic lesion in cats and monkeys regularly at velocities at impact of 28 feet per second. It was necessary to have a striking piece of weight at least equal to that of the head of the animal, and since fracture of the skull is not desired in these experiments it is necessary to use wide striking area and choose a region of skull buttressed by thick bone, as in the occipital region. A threshold of 29.4 feet per second was reported by Gurdjian and Webster (40) and of 30 feet per second by White *et al* (101). Walker *et al* (98) in an illustration claim that "momentary concussion" was produced at a velocity of 9.6 feet per second. The physics of such an impact are, however, complex and the factors of mass and speed of blow are only of importance in relation to their effect on the head of the animal, for unless a depressed fracture is caused, it is the acceleration of the head that is the essential factor. In the absence of any satisfactory way of measuring this, the speed of applied blow is an approximation which is of value only as long as the effective mass of the striking force is greater than that of the head, and is varied to some degree by

the elasticity of the skull and cushioning of the scalp. Such factors also presumably explain the greater ease of inducing concussion by occipital blows in the sagittal plane than in other planes, though the difference between the effect of striking speeds is relatively slight.

It must be stressed that effects are induced by blows of lesser velocity, notably a brief but sharp fall of blood pressure and a start reflex or sudden flexion of all limbs with inspiratory gasp. This latter reflex can be induced by a blow to the neck or upper thorax in a lightly anesthetized animal. The corneal reflexes are not immediately lost, though near the threshold for concussion a transitory impairment is often found. If a level below 50 mm systolic blood pressure is recorded for 10 to 20 seconds some delayed impairment of reflex responses may occur. In the decerebrate animal acceleration of respiration is prominent. Denny-Brown and Russell called these "subconcussive effects," attributable to stimulation of the vagal mechanism. They considered it the probable mechanism of the "knock-out" in boxing where the subject sags gently to the floor without retrograde amnesia and with relatively rapid recovery. Concussion does occasionally occur in boxing but the subject is then "knocked stiff," lies pale and immobile and has no memory of the incident. Williams and Denny-Brown (103) found no electroencephalographic changes in subconcussive effects in cats. Dow and his associates (22) have investigated 74 cases of human head injury, seen immediately after the accident, in which the speed of fall or speed of striking object could be computed with reasonable accuracy. They found that patients who had traumatic amnesia and abnormal electroencephalographic records had suffered impacts at an average speed greater than 24 feet per second, while those who had not lost consciousness and had normal or borderline electroencephalographic records had an average velocity of impact less than this. The limits of the exceptions to the general rule where patients who had been concussed at an estimated speed of blow as low as 16 feet per second, and others who had not been clearly concussed after an impact at 56 feet per second. The effective mass at time of impact is a more difficult matter to compute and may have accounted for some of the exceptions. It is also difficult to find a sharp limitation of electroencephalographic change due to focal injury from that due to concussion. The average speed therefore appears of greater significance than the limits encountered.

All the phenomena of such experimental "acceleration" concussion in the cat, except the electroencephalographic change, could be induced in the decerebrate or decorticated animal, then being free from the complication of anesthesia. The stalling or stunning of an active preparation is then regularly accompanied by loss of corneal reflex, by rise of blood pressure followed by deep and prolonged fall, by respiratory gasp and start with prolonged apnea in inspiration, and by absence of other reflexes (swallowing, knee jerk) for an interval. The course of recovery of each function was gradual. In both decerebrate and intact anesthetized animals it was found that a peripheral stimulus was without effect on blood pressure or respiration until late in recovery. Transient dilatation of the pupil was common as a delayed event, as was also

a delayed secondary rise of blood pressure, for which adrenalin may be responsible. Denny-Brown and Russell used these observations in making their hypothesis of the mechanism of the recorded events. They considered that the cycle of events in the respiratory and vasomotor centers was a pattern of the disturbance of neuronal function. They pointed out that all mechanisms may show evidence of both initial and continued stimulation (inspiratory gasp, rise of blood pressure, closure of lids, twitch of vibrissae) which indicated that the lower levels of function continued to be stimulated but were inaccessible to reflex stimulus during the duration of concussion. The duration of areflexia varied in each case, being shortest for respiration, longer for corneal reflex and vasomotor effects, and longer still for the pinna reflexes.

Some difficulties are encountered by this hypothesis. First that a convulsive gasping spasm regularly appeared in lightly anesthetized or decerebrate animals with a latency of 5 to 10 seconds after the impact. It had been found that a strong stimulus could break through the reflex block at an earlier stage of recovery than could weaker stimuli. The delayed motor disturbance, often coinciding with delayed onset of heart block, was regarded as the result of residual powerful afferent excitation remaining from initial direct stimulation of the 10th nerve roots by the injury, manifested at the earliest recovery of reflex block. Secondly, the rise in blood pressure commonly gave way to a steep fall, which sometimes proved fatal. This diphasic curve was explained as a stage of exhaustion of the vasomotor center after its initial discharge and before recovery of the reflex compensatory mechanisms. The presence of continued discharge of the center was demonstrated by the ability of a sudden passively induced rise of intracranial pressure to cut short the rise in blood pressure at any stage. Gurdjian and Webster (40) observed that the rise of blood pressure is prevented by spinal transection or by the use of yohimbin to inhibit adrenalin. Walker *et al* (98) found continuation of hypertension after spinal section but do not appear to allow for the stimulating effect of such section or the stimulation by adrenalin already in progress. Lastly there remained the difficulty in accounting for the occurrence of a simple fall in blood pressure and simple cessation of respiration with loss of all reflex and motor behavior following a severe blow in deeply anesthetized animals. Here the anesthetic was considered to have impaired reflex function and reaction and the trauma to have had a purely or almost purely paralyzing effect. Hemorrhage has the same effect, as noted by Pols and ourselves. Thus it was proposed (Denny-Brown and Russell, 15) that the complicated sequence of effects of brief physical trauma could be resolved into four degrees: excitation, excitation with reflex paralysis, excitation followed by complete paralysis, and complete paralysis alone (under deep anesthesia). The stage of paralysis of whatever kind was synonymous with concussion, and was reached at a threshold intensity which in the case of the movable head could be measured in terms of a threshold velocity at impact.

*"Compression" concussion* Denny-Brown and Russell (15) drew a sharp distinction between the changes produced by a blow to the freely movable head and the effect of a sudden brief rise of intracranial pressure. They demon-

strated that passively applied pressures of 50 to 100 mm Hg lasting 1 to 5 seconds had an effect which involved a prolonged fall of blood pressure, and initial acceleration of respiration followed by prolonged and often fatal apnea. This effect was uncertain, and similar or greater pressures often produced no effect. Herniation of the cerebellum and medulla at the foramen magnum was found in fatal experiments. On the other hand, an exceedingly brief impact of increased intracranial pressure induced by a blow on the plunger of a syringe evoked a concussion with loss of corneal reflex followed by gradual recovery, transitory immediate apnea, and small rise in blood pressure. As compared with "acceleration" concussion, the rise of blood pressure and the respiratory disturbance were slight in relation to the duration of loss of corneal reflex. The pinna reflex was found to be longer delayed in recovery than the corneal response. Reference to the records of Kocher (59), Cannon (9), Knauer and Enderlen (57), Scott (90) and others who, by striking the fixed skull, had used an injury which induced compression, revealed a similar absence or smallness of respiratory and cardiac response, as compared with those of Polis (81) and ourselves in accelerative injury. The condition resulting from very rapid brief compression was therefore distinguished as "compression concussion." It was pointed out that this also had a threshold value for elicitation which could be expressed in speed of application, though no attempt to measure it was made. By means of fitting to the pendulum a small piston which fitted a trephine opening in the fixed skull, and arranging the pendulum to bounce back from a metal stop after the piston had penetrated a regulated distance, we (15) showed that very severe compression concussion could cause intense apnea and prolonged rise of blood pressure, then indistinguishable from acceleration concussion, though small contusions of the brain stem were a common complication.

It is important to exclude the effect of herniation of the cerebellum at the foramen magnum and the production of lesions in the lower medulla and upper spinal cord in this way. Thus Githens and Meltzer (32) report as "concussion" with "unconsciousness," a condition of quadriplegia without loss of corneal reflex produced by means of a weight falling on the supported head, and associated with hemorrhage into the medulla and upper cervical cord.

Groat, Magoun, Dey and Windle (38) and Walker *et al.* (98) have since compared concussion produced by acceleration and by compression and found no essential difference in the sequence of physiological changes to result. Applied transient increase of intracranial pressure was found to have a threshold value expressed in total energy by the former authors, who also noted greater effectiveness if the cannula were connected to the posterior fossa. No studies of speed of application have been made. Gurdjian and Webster (40), (100) had difficulty in producing concussion uncomplicated by hemorrhagic lesions in dogs, but found no essential difference in the phenomena of response to a blow by pendulum to the movable head, the fixed head, or by penetration of bullet. In each case the degree of response could be graduated in terms of energy of striking force. Brief compression by transmission of fluid impact is therefore a convenient and reliable

method of inducing concussion. This does not however mean that the mechanism of "acceleration" concussion is necessarily identical with the effects of brief compression, or that concussion is the effect of a brief anemia of the brain. The hypothesis of Duret (23) which assumes that a wave of cerebrospinal fluid distends the ventricles, and related explanations, do not have further consideration since it has been demonstrated that concussion can occur after drainage of all fluid, and indeed in a decerebrate preparation when the cerebral ventricles have been removed.

*The physiological mechanism underlying the generalized response.* The experiments of Witkowski (107), Pols (81), Miller (68), White *et al* (101) and ourselves have clearly shown that the mechanism of the physiological effects is independent of the occurrence of meningeal or petechial cerebral hemorrhage and of cerebral or spinal contusion. Examination of nerve cells of animals dying from such concussion or killed within two hours revealed no changes. The blood vessels of the brain are histologically unaltered. Denny-Brown and Russell (15) and White *et al* (101) failed to find evidence of fat embolism which Gutiérrez-Mahoney (43) had claimed. Windle, Groat and Fox (106) have more recently also failed to find any histological change in such animals with the same methods by which they have demonstrated the remarkable delayed histological change which will be discussed below. Denny-Brown and Russell (15) were able to demonstrate that when petechial or meningeal hemorrhage or contusion did occur they developed in the course of one or more minutes following the injury when the chief effects of concussion had already subsided. The effects produced by such hemorrhages were traceable to any increase of intracranial pressure caused by them. Sensitive recording of intracranial pressure revealed no change in the first hours following concussion without lesions, except for a transitory rise of 2 to 3 cm. of water accompanying the immediate rise of blood pressure (15), or a slightly greater increase, attributed also to vascular congestion by Pilcher (80). Delayed effects, such as these described by White *et al* (101), are not of account in the immediate phenomena.

If discrete lesions of the brain and cerebral edema are excluded as the basis for the immediate phenomenon of concussion there is next to be considered the possibility that the condition is determined by primary disorder in one part of the brain whence some secondary reflex or other effect affects the remainder. Concussion is mimicked to an extraordinary degree in the stilling of automatic behavior of the decerebrate "midbrain" animal. The disturbance of the medullary centers is then identical with those of the intact animal. Section of the VIIIth nerves excludes labyrinthine shock as a primary stimulus. The electroencephalogram has however given strong evidence that the cerebral cortex is primarily affected, independently of the bulbar centers. Williams and Denny Brown (103), in cats lightly anesthetized with nembutal or ether, succeeded in showing a great loss of voltage in the electroencephalogram with progressive but slow recovery of wave forms. This change was independent of the apnea of concussion. Following abolition of the corneal reflex for 19 seconds in one ex-



periment the electroencephalogram showed only small voltage spikes for 7 seconds, very low voltage for 2 minutes, and fully recovered only after 20 minutes. Two and one-half minutes after the blow slow waves at one electrode began to be prominent and remained so for some minutes. These very slow waves have not been commented upon by other investigators, though they are prominent in prolonged traumatic confusion (Williams, 1952) and may represent this stage in more brief concussions. The effect was generalized, though the blow was given on one side of the head. If a heavy blow were given at one point in the skull, over one electrode, and the head prevented from moving, an identical but brief loss of voltage and progressive recovery occurred under that electrode and not elsewhere. These investigators therefore made a further point, that the electroencephalographic effect of physical impact can be localized, and the phenomenon seen in cerebral concussion is particular only in its generalization.

Dow and Raaf (1952) had an exceptional opportunity for observing early human electroencephalographic changes in head injuries in shipyard accidents. Patients who had suffered an amnesia following the trauma but were clear mentally by the time the tracing was taken showed only a slight increase in the percentage of abnormal records as compared to a control series without loss of awareness. If there was impairment of consciousness of any degree at the time of the electroencephalogram the tracing was abnormal. The electrical changes are thus brief in most cases. In all cases the abnormality in electrical record was less reliable than clinical judgment in predicting time lost from work. Jasper *et al.* (1956) and Williams (1952) have studied the prolonged electroencephalographic changes that accompany prolonged traumatic confusion, and the latter author has pointed their resemblance to those of transient mild injury, and has followed their subsidence. They do not appear to be related to post-traumatic epilepsy, which is closely correlated with localized cerebral laceration (1952).

Govons, Lewey and Grant (1956) and Govons (1955) recently reported a disorder produced by the explosion of a detonator attached to the parietal vertex in dogs, which differs considerably from the effects already mentioned. After an initial period of loss of reactivity lasting some minutes there was very slow recovery of reflex and general responses, after which period gross ataxia of gait and station became apparent and lasted 1 to 10 days. For days the animal was unable to land without falling, following a jump from one level down to another. The condition was reproduced in many animals, and in 4 of 12 surviving dogs no hemorrhages were demonstrable in the brain stem or cerebrum. A similar condition in a human patient was also described. Whether the ataxia was the result of parietal, cerebellar, or labyrinthine disorder is not clearly indicated by the experimental evidence, but the authors favor explanation by labyrinthine lesion. The labyrinths are to be reported upon later. It would appear necessary to postulate some localized intensity of the injury, so as to confine the most intense disturbance to one physiological mechanism. We have seen that the electroencephalographic change can be localized to one area of the cortex by a localized injury, and there would appear to be no *a priori* reason against localization of more severe or more persistent damage to one or other part of the brain by some

peculiarity of stimulus. It will be apparent however that in cerebral concussion, as generally understood, there is no evidence of localized effect.\*

The possibility that generalized cortical changes in concussion result from some temporary ischemia of the cerebral cortex has been the basis of many hypotheses (59), (97), (90). The measurement of blood flow through the brain during and immediately following a blow to the mobile head offers a considerable technical difficulty. Methods involving drop recorders of jugular flow further involve the secondary effect of the loss of blood. With such recorders Polis (81) and Knauer and Enderlen (57) recorded a large increase in jugular flow accompanying the phase of rise of blood pressure, followed by diminished outflow. The former investigator provoked the injury with the head mobile, the latter with the head fixed. This was confirmed by Denny-Brown and Russell (15) by the same method with "acceleration concussion" and in addition these investigators found an increased outflow accompanying the small fall in blood pressure of "subconcussive blows." This increase in cerebral blood flow, associated with bradycardia and pause in respiration, appeared to derive explanation from stimulation of the depressor mechanism of the vagus by subconcussive blows, and has no essential part in concussion. With such a method a momentary pause commonly found before the increased rate of flow begins may have been a purely mechanical effect of the blow. The increased outflow was more prolonged in concussion when vagal effects are in any case also more prolonged. Polis found a momentary fall in blood pressure in the peripheral end of the cut internal carotid in some experiments, from which he postulated an initial spasm of the circle of Willis. This we were unable to confirm. The decreased cerebral blood flow one hour after trauma with decreased oxygen consumption, found by Lindquist and LeRoy (65), was in the presence of cerebral contusion which is itself a demonstrable alteration of cerebral circulation and should not be allowed to complicate discussions of cerebral concussion.

Reports of decreased oxygen saturation of arterial blood by Schnedorf *et al* (89) following "brain concussion" take no account of the respiratory changes involved, and do not define criteria of concussion except to note that unconsciousness in one dog lasted two minutes, and that in all animals except one the spinal fluid was blood stained. Gurdjian and his colleagues (41) have recently made a careful chemical analysis of the brain, finding no change in cerebral  $\text{pH}$  differences in oxygen, carbon dioxide or glucose content. They found an increase of oxygen saturation of arterial blood proportional to the disturbance of respiratory function. Their experiments were complicated by contusions but they note that some areas of the cortex were chemically normal even in profoundly injured animals and that the changes indicative of defective oxidation occurred in areas of marked contusion where they were proportional to the degree of local damage. The report of Krauer and Enderlen (51) of increased acidity of traumatized

\* Labyrinthine concussion as judged by the appearance of immediate and severe vertigo with progressive subsidence of that symptom is a very unusual accompaniment of head injury and the post traumatic dizziness commonly complained of is thought to have other origin (Friedman, Brenner and Denny Brown 30)

cortex to litmus is presumably related to the appearance of lactic acid, but its absence in uncontused cortex in the careful work of Gurdjian *et al* indicates that it need no longer be taken into account in the mechanism of concussion

It therefore does not appear possible that the cortical phenomena of concussion can be explained by the accompanying disorder of the bulbar mechanism. Indeed the immediate onset of unconsciousness and the peculiar phenomenon of retrograde amnesia completely differentiate concussion from syncopal attack or anoxia. The only comparable reaction in man is the confusion, often with retrograde amnesia, induced by cerebral electric shock, as now widely used in psychiatric therapy

The electroencephalographic method as used by the author did not however allow any observation of the immediate electrical effect owing to blocking of the amplifiers for ten seconds by the impact contact. Walker, Kollos and Case (98) have endeavored to overcome this difficulty by the use of a voltage divider to stabilize the amplifiers. Concussion was induced by the fall of a weight onto water in contact with the dura mater in cats. They found a large spike potential (approximately 1 millivolt) to accompany the impact followed by a series of very fast spikes of the kind commonly seen in muscular activity for 10 to 20 seconds, and this in turn by a relative inactivity for a further period of seconds or minutes. Thereafter there was slow return to normal electroencephalographic activity. The animals were curarized in order to avoid the start reflex. An initial spike potential up to a hundred microvolts was found with the first repetition of the blow after death, thereafter no change. The effect of lesser blows than that estimated to cause concussion is not stated, nor is the early fast electroencephalographic discharge correlated with the other physiological changes. These investigators maintain that the primary event in concussion is an intense excitation of the nervous system, with activity which they term "after discharge" lasting up to 20 seconds, followed by a phase of decreased activity with a raised threshold to excitation which they categorically identify with "extinction". The disorder of consciousness is explained by analogy with the similar disturbance accompanying electrically induced convulsions in man. This view appears to be an oversimplification of the problem, for although a generalized excitation at the moment of impact occurs in lower level systems, and is likely in the cerebral cortex, the sequence of events from that point onward is more complex. A convulsive spasm is commonly seen in animals but after an initial flaccid delay of 5 to 10 seconds from the moment of impact. It is moreover usually a burst of running movement or intense general flexion, and tonic-clonic phases characteristic of cortical activity have not been seen by ourselves, though they are prominent in cerebral anoxia. In man there is no record of a convulsive fit with concussion, though the author has observed generalized extension of the limbs for a few seconds, with latency similar to animal experiment. An additional difficulty in the interpretation of the records shown by Walker *et al* (98) is that the method of induction of "concussion" is such that it is necessary to eliminate residual compression. The magnitude and duration of pressure change from the

falling weight are not stated.<sup>4</sup> It would appear important to exclude prolonged ischemia of the cortex. The anesthesia, as Williams and Denny-Brown (103) remark, has to be extremely light. If the anesthesia were deep it was difficult to produce any prolonged effect on the electroencephalogram, although the paralytic phenomena of concussion on brain stem mechanisms were more severe and less complicated by excitation. There is no convincing evidence that "intense traumatic stimulation is followed by the same electroencephalographic, chemical, and clinical phenomena which characterize intense stimulation of the nervous system by electrical, chemical or other agents" (98), for in man convulsive effects are prominent in all these except trauma. Lastly, it may be likely that the neuronal disturbance of concussion is a universal phenomenon in nervous gray matter, and a phenomenon comparable to "extinction" arising from a single stimulus has yet to be demonstrated in medullary centers.

One of the most enigmatic features of concussion is the sensitivity of supranuclear mechanisms to the condition. Denny-Brown and Russell (15) in attempting to account for the complex sequence of events in the recovery stage postulated a paralysis of reflex transmission as explanation. Groat, Magoun, Day and Windle (38) by ingenious placement of electrodes by the Horsley Clark instrument succeeded in measuring steady thresholds of excitation for brain stem nuclei, supranuclear pathways and tracts. They found that either acceleration or compression concussion sufficient to abolish the corneal reflex raised the threshold momentarily in motor nuclei, and very considerably and for long periods in supranuclear pathways. Thus for basis pedunculi (white matter) there was no change in threshold during concussion but for motor cortex a rise in threshold from 3.5 volts to 7.5 volts. For facial nerve fibers near the genu there was no change but for the same movement (lid closure) from the neighborhood of the facial nucleus a sharp immediate rise from 0.8 volt to 2.5 volts was found. The effect was transitory, but could persist for as long as 1½ hours before full recovery. It would thus appear that heightened reflex threshold is a general phenomenon, and the exact measurements of Groat *et al* give sound basis for the hypothesis that each "center" is affected in the same way, but for a period which bears a fairly constant relationship to other centers. The transitory disappearance of the knee jerk and lower level reflexes no doubt reflects a similar change in the pontine reticulum. In very severe concussion the threshold of lower motor neurones was raised.

The physiological evidence appears now well enough established to conclude that the cerebral phenomena of cerebral concussion are the result of a cycle of events in each and every neurone mechanism within the cranium. The process is characterized by excitation followed by a refractory interval which varies from neurone to neurone, being most brief in the respiratory center and most prolonged in some portions of the cortical apparatus. Further, it appears to be

<sup>4</sup> Doctor Walker has informed the author that the significant pressure change lasted approximately 0.1 sec., and that a residual pressure of not greater than 2 to 3 cm. water remained.

the posterior fossa had been decompressed, and a correlation of degree of disorder with velocity of bullet. The 22 calibre bullet is relatively large compared to the size of the intracranial cavity of the dog, so that the size of projectile should be added to the factors they mention. The mechanism of action of such medullary disorder is that we have called compression concussion.

The question of movement of intracranial contents in head injury has been settled by non-instrumental means. In a series of experiments brilliantly conceived and as brilliantly executed a group of investigators—Sheldon, Pudenz, Restarski and Craig (91)—have devised a means of replacing the whole upper half of the skull of monkeys by a lucite calvarium. The dura is removed widely on either side of the sagittal sinus so that the brain can be observed in life. By means of high speed photography and a pneumatic plunger which produced graduated impact the behavior of the brain in concussion, and the effect of various directions and velocities of blow have been analyzed. In such a film shown by these investigators at the Annual Meeting of the American Neurological Association (1944) the effect of a lateral, temporal blow was seen to cause an indentation of the part struck followed at a perceptible but very small interval of time by sudden forward movement of the opposite side of the skull. The brain appeared to contract proximally with the impact and expand distally with the forward movement of the distal portion of the skull, following which a distinct but much slower swirling commotion of the brain within the cranial cavity occurred. These experiments also indicated that no remarkable anemia of the brain was part of the process and that considerable vasodilatation followed concussion. These investigators will report a detailed analysis of this phenomenon in due course, but for present purposes it may be taken that the rapid physical phase is a condensation of the whole brain, that is compression, then expansion, followed by movement about its attachments. Thus two possible physical mechanisms may occur.

It is remarkable that in acceleration a fall of pressure at one side of the brain and a rise of pressure at the other should produce a generalized phenomenon. Theoretical consideration of the hydrostatics such as those advanced by Goggio (33) do not offer assistance in this problem. Holbourn (50) has maintained on theoretical grounds that shear stress is more important than direct hydrostatic effect in the causation of concussion, and supports his contention by indicating the greater tearing of a gelatine model at points of partition when the model is rotated. Though this hypothesis has certain plausibility in relation to the common situation of pial contusion at the frontal and temporal poles in head injury, its author ignores certain important considerations. The most important of these (76) is that rotation of the head about an axis passing through the skull would be a peculiar origin of head injury, and that when the axis of rotation is outside the skull shear forces are minimal. Further, concussion in our experiments still occurred if the movement of the head was minimal (3 mm) and purely postero-anterior. Finally, shear forces, by their tearing effect, must have predominant effect on the supporting vaso-astral framework of the brain, and it should now be clear that no case can be made for vascular lesions as a basis of concussion.

It is to be hoped that some means will be found to study the effect of distortions of structure (whether by rotational or linear shear stress) apart from circumstances of high speed compression-rarefaction, for there is now considerable evidence that all types of vascular lesions (including petechial hemorrhage) are independent of concussion both in causation and in effects. The effects of two types of movement seen through the lucite calvarium (91) could then be resolved with more certainty.

It is time that discussion of the mechanism of concussion be cleared of the confusing issues introduced by the focussing of attention on intracranial pressure by Kocher. We have maintained that compression concussion and acceleration concussion must have some common element of physical stress, although in the latter the pressure on one side of the brain falls at the moment of increase on the other. The factor common to both is condensation of the substance of the brain. All the evidence at present available supports a hypothesis by which concussion is a reaction to physical condensation of nervous tissue. Such condensation, like other kinds of nervous stimulus, requires a certain threshold rate of change to be effective.

*The effects of the "blast" of explosives.* The possibility that explosive force transmitted by air could induce some physical change in the central nervous system identical with or allied to cerebral concussion has been debated for a very long time. Accounts of military medicine in Napoleonic times discuss the possibility of some injury to the brain arising from the "wind" of a cannon ball close to the head (44), (21) and the discussions concerning "shell-shock" and "windage" in the World War 1914-18 are well known. Today the same subject is revived under the name "blast concussion" and Fulton (31) has given a general review of the problem. The term "shell shock" has been largely discontinued owing to the recommendations of a British War Office Committee (99) and a Ministry of Pensions Committee (69). These bodies recommended that clinical cases thought to be suffering from the transmitted effects of an explosion should be classified under recognized diagnoses such as psychoneurosis, cerebral contusion, concussion, etc., as became such states after more usual forms of head injury. There are many disadvantages that accrue from the loose application of a term with uncertain and even mysterious connotation in the eyes of the soldier (19).

At the outset of any discussion it should be clear that persons closely exposed to any explosion are liable to be injured by being thrown against some solid resistance, or by being struck by flying debris or portions of the explosive container. Thus, of a series of such cases, Eden and Turner (25) were able to account for all instances of unconsciousness in terms of such secondary injuries. The writer has related case histories from his own experience of exposure within 12 feet of bomb explosion with full memory of the injury although particles had lodged in the brain (17). In another case with a hysterical amnesia recoverable under amytal hypnosis the patient had been thrown into a heap of soft lime by the explosion. Both these varieties of injury are common within the experience of military surgeons. More difficult to assess are patients found wandering in a state of shock and confusion after exposure to an explosion without ex-

of injury and with resistant amnesia for the event. Unfortunately, concussion is often sustained without sign of external injury in more usual circumstances such as a fall on a sandy surface. If such a condition as concussion from explosive force alone is to be established it is absolutely necessary to have full documentation of cases where all possibility of secondary injury is absent and amnesia both retrograde and immediate is verified. No such cases are on record.

The oft cited cases of Mott (71) do not withstand analysis, for the most convincing had been buried by an explosion, and the petechial hemorrhages found in the brain may have been due to CO poisoning from the fumes of explosion. In a further report Mott (72) cites two further cases. One of these had died suddenly following twenty-four hours of confused excited behavior, after exposure to many shell bursts. Multiple superficial petechial hemorrhages were found on the surface of the brain and there was a bruise of the scalp in the frontal region which suggests secondary injury. There was also a possibility of CO poisoning. The second patient had been sitting in a hut close to a large explosion and consciousness was not regained before death 12 hours later. Superficial hemorrhages were found over the cerebellum, temporal poles and one frontal lobe, with early and doubtful chromatolysis in the frontal cortex. The possibility of secondary injury was not excluded in this case also, and the changes found were those commonly seen in gross cerebral contusion. An interesting observation by Wilson and Tunbridge (105) on the occupants of a deep cave in Malta, at the entrance to which an aerial bomb exploded, describes the immobile apathy of the surviving occupants but gives no details of their disturbance of consciousness. The pathological observations are concerned with the pulmonary injuries alone.

The subject has been approached experimentally by a number of investigators. Carver and Dinsley (10) observed the effect of underwater explosions on fish, and describe a state of suspension of nervous activity which ended fatally in those in immediate proximity, and was followed by recovery after intervals up to 12 hours in those at a larger radius. At autopsy fish killed by the explosion showed petechial hemorrhages in the central nervous system, gills and viscera. Rats and mice were exposed to explosions in air, and animals showing no sign of direct trauma due to fragments of cage were rendered unconscious with bleeding from the orifices. "Varying amounts and degrees" of internal hemorrhage in the viscera and central nervous system were revealed. Some animals were found dead without visible macroscopic abnormality except general capillary engorgement especially noticeable in the nervous system. Animals exposed at greater distance were found in a state of "stupor," frequently with twitching of the limbs on recovery and exaggerated reflex activity. These animals also did not have lesions visible to the naked eye.

Marnesco (67) reported briefly on animals exposed to explosives, noting that there was no loss of consciousness, but that hemorrhage from the nose and mouth and ataxic gait was common. Petechial hemorrhages were found in the brain and spinal cord in many animals and the pulmonary lesion was well described.

Mauret and Durante (66) exposed rabbits to explosions in air. The animals

were either placed in trenches or suspended in air. Animals that succumbed were found to have pulmonary hemorrhages, small subpial hemorrhages on the spinal cord and nerve roots. The brain was without lesions in most instances but showed some petechial spots on the surface or in the pons. Those that survived showed acceleration of respiration and transitory myosis, and some were excited, some apathetic. The cells of the cerebral cortex were normal. After survival for long periods some unconvincing lesions were found, thought by the authors to indicate healed vascular lesions.

The very careful experiments of Hooker (52), (53) deserve particular mention. He exposed dogs at various distances to charges exploded on open ground, and to the muzzle blast of large rifles and howitzers. He describes the state of immobility and lack of reaction which these animals showed for periods of 4 to 6½ hours following the exposure. In no instance were the eyes closed, or reaction to stimulus abolished. The most striking effect was the sudden onset of "extreme fatigue." An animal previously alert, active and normal in every way was promptly changed into an exhausted lethargic animal which roused when spoken to or touched "and would move about heavily for a few moments at a time."

He likened the condition to surgical shock and measured the severe fall in blood pressure (to circa 50 mm) found in every animal so examined. The abdominal veins were found to be distended at autopsy, but in no single instance was there evidence of macroscopic central nervous lesion. Fat embolism was sought for and not found. Pallor and other evidence of shock are commonly recorded immediately after exposure to explosion, and the experiments of Hooker indicate that this is not necessarily to be explained on an emotional basis. Hooker found pulmonary hemorrhage invariably associated with the condition of shock he described but under some circumstances when the pulmonary lesions were maximal the shock was least pronounced. He was therefore unable to derive an absolute correlation between the conditions. It is possible however that sudden traumatic compression of the thoracic cage is a strong vagal stimulus and no experiments to explore this possibility have to the writer's knowledge been made.

Zuckerman (108, 109, 110) and Krohn, Whitteridge and Zuckerman (62, 63) have repeated exposure of animals to air blast and have described the pulmonary lesions in detail. In goats and monkeys no cerebral lesions were found after exposure to blast pressures as high as 110 lb per square inch (110). Though a ventricular or subpial cerebral hemorrhage was found in some rabbits, no condition of immediate traumatic unconsciousness was observed. Extradural spinal hemorrhage, and hemorrhages in spinal nerve roots were more common. In isolated instances of cerebral lesion the possibility of secondary cerebral injury from contact with the ground or cage, or of some embolic lesion from the pulmonary clots cannot be excluded. This difficulty also occurs in the report of a pheasant found in a dazed condition near a bomb crater by Stewart, Russell and Cone (92), where autopsy disclosed numerous petechial hemorrhages throughout the brain. Pathological reports on human cases dead of exposure to explosive



blast (26, 45, 75, 109, 63, 46, 47, 105) have all focussed attention on the pulmonary condition. Neither clinical nor autopsy evidence has indicated damage to the central nervous system. In only one case of blast injury, fatal in 4 hours, has autopsy revealed small submeningeal hemorrhages over the brain with a little blood in the ventricle (74). The immediate state of this patient was obscure for he appeared to have been asleep inside a hut which was demolished by the explosion.

The occurrence of visceral lesions resulting from exposure to underwater explosions has focussed attention on waterborne blast effects. A number of reports on clinical and pathological material are available (8, 34, 77, 48). Of these only Hamlin reports neuropsychiatric sequelae. In 35 cases examined by him one died of subdural hematoma which was however considered due to some local injury, probably from an earlier direct injury before immersion, one was disoriented with bloody spinal fluid, possibly from the same cause, and in the others there appears to have been no retrograde amnesia, though 4 of these suffered from some delayed loss of consciousness. None of the cases recorded by Goligher (34) lost consciousness or showed signs of damage to the nervous system.

The possibility of transmission of the impact of waterborne blast to the nervous system by the blood vessels (71, 92), or by the spinal fluid (48) has been suggested. Animal experiment (104, 37, 29) on goats and guinea pigs exposed to underwater explosions failed to demonstrate any evidence of central nervous lesions, and the animals were not rendered comatose immediately following injury. Observation of the reflex, cardiovascular and respiratory responses throughout such exposure was naturally difficult, and Clark and Ward (12) and Clark (13) overcame this difficulty by the use of a U-shaped tank, in the one limb of which the animal was immersed, in the other a float which could be struck by a falling weight. By this means these investigators demonstrated the mechanism of pulmonary and visceral lesions by the impact transmitted through water, even if a metal barrier prevented actual surge of fluid. Such an impact presumably travels with the speed of sound, and is of an estimated one five-thousandth of a second in duration. The mechanically induced wave appears to reproduce the effect of explosion in air or water, where transmission is of the same order. Hemorrhages visible to the naked eye did not occur in the nervous system. "Although occasional animals showed loss of corneal reflexes, ataxia and incoordination immediately after the blow, it was exceptional that any of these signs suggestive of direct and immediate involvement of the central nervous system occurred." No electroencephalographic evidence of concussion was found. Death could however be induced within 2 to 3 minutes. The impact was followed immediately by cessation of respiration and great slowing of the heart, effects which were abolished by vagal section. Re-establishment of labored respiratory movements within a minute was followed by gradual failure of respiration and heart beat if death occurred. Clark and Ward have thus established the chief characteristics of pulmonary and abdominal disorder by sudden impact. The close resemblance of the pulmonary and cardiac disturbance to that of cerebral concussion is to be noted. They investigated the effect of

immersing the head of the animal with and without exposing an area of dura, but were unable to induce cerebral concussion in rats or cats by this means. They remark that perhaps greater impacts might have induced cerebral concussion but since animals died within a few minutes of pulmonary hemorrhage with the blows used the problem of the influence of cerebral concussion was of minor importance.

The problem of cerebral concussion in blast injuries has therefore greatly diminished in importance following these clinical and experimental observations. Sudden death or coma following exposure to blast in air or water no longer necessitates explanation by a central nervous effect. Experience has revealed that loss of consciousness is delayed and not accompanied by retrograde amnesia. There remains however the occasional occurrence of petechial hemorrhages in both human and experimental observations (Mott, Marinesco, Carver and Dinsley Stewart *et al*, O'Reilly and Gloyne), or blood in the spinal fluid (Hamlin), and the occasional immediate loss of reflexes (Clark and Ward) or apathetic stuporous state (Hooker, Wilson and Tunbridge) or transient ataxia (Marinesco, Williams, Clark and Ward), to be explained.

Petechial hemorrhages in the brain can be caused by direct injuries but are then not usually generalized, as in Mott's cases, where the question of carbon monoxide fumes from the explosion was raised. Traumatic asphyxia, when widespread cutaneous, ocular and retro-orbital petechial hemorrhages are commonly found as a result of severe compression of the chest for 1 to 5 minutes (49, 5), and have occasionally been found in the brain in some cases where consciousness was not lost, appear to the writer to be a possible explanation in Mott's first case, where the patient was buried by the explosion, and perhaps in other cases of pulmonary contusion. The absence of cutaneous lesions would require some modification of the usual hemodynamic mechanism. Small terminal hemorrhagic spots within the nervous system are not uncommon in death from congestive heart failure and, as in the condition called traumatic asphyxia, the severe embarrassment of the venous circulation following pulmonary blast lesions may have a purely mechanical effect separable from the impact of the explosion. Robb-Smith (82) found fat embolism in some cases of severe multiple injuries from debris of bomb explosion, but these patients did not clinically resemble "concussion." An interesting case reported by Ascroft (2) presented two larger lesions which appear to the present writer to have been infarcts of the brain, as well as multiple petechial hemorrhages, following explosion of a "booby-trap" held against the chest wall. No trace of fat embolism was found. This case appears to raise a clear possibility of multiple emboli from the damaged lungs in these conditions and this should also be investigated. Hemorrhages in the spinal cord and roots have a more obvious explanation in distortion of the spine by the injury and fracture of the spine (29) or paraplegia (64) have been observed in experimental animals. The negative pressure wave of blast may be supposed by some to have some particular effect, but this has been demonstrated by Latner (64) to induce the characteristic pulmonary lesion.

We have seen that the definition of cerebral concussion includes an

loss of awareness It is naturally difficult to exclude the absence of a momentary loss immediately following a devastating explosion The state of apathy so clearly described in Hooker's animals however appears to allow no such possibility and was in them attributable to the related fall in blood pressure Clark and Ward do not remark upon the blood pressure in their animals, but the other immediate effects referable to strong vagal stimulation, followed by severe obstruction of the circulation, presuppose a cerebral anoxemia of such rapidity and severity that loss of reactivity could well occur in some animals within three or four seconds of the impact The adrenals were not histologically changed (Marinesco) The ataxia and inco-ordination described in animals were not such as to require necessary damage to a nervous pathway The whole disturbance appears to fit a description of acute circulatory collapse or shock

Concussion, or indeed any similar physical damage to nervous tissue resulting from the force of an explosion transmitted by air or by water, therefore remains an unproven condition Though high acceleration of the head by explosion would appear to be possible, the fact that explosive blast is a movement of air and that such air has to accelerate the heavier, rounded head warns that the initial acceleration in the critical first half-millisecond is not comparable with that produced by a solid mass moving at a much slower speed Explosive blast exerts its full effect when mass is small in proportion to surface area, otherwise it is deflected

#### CONCLUSIONS

Cerebral concussion is a phenomenon which has not only important practical considerations, but also merits attention as a fundamental mode of reaction of nervous tissue Though recent investigation has done much to clarify its essential nature there are many aspects of the subject which present unsolved problems Some of the factors that determine the presence or absence of concussion in penetrating gunshot injuries to the brain are now explained The separation of the effects of concussion and of multiple hemorrhages, and the greater understanding of the pulmonary lesions due to explosive violence have seemed to clear up many of the doubtful features of the effects of explosive blast No convincing evidence is found to support the hypothesis that explosive blast can produce a cerebral lesion comparable to concussion

The introduction of methods of reproducing standard degrees of the condition and of measuring both the quantity of injury inflicted and the amount of effect produced have opened the possibility of measuring its relation to nervous metabolism Its effect on synaptic transmission and the delayed development of a cycle of histological changes are at present complete enigmas which invite investigation Its relationship to shock, to fatigue and to the similar but different changes induced by anoxia are unexplored The cumulative effect of repeated minor concussion would appear to offer a good starting point in such investigations

It is maintained that in all such investigations the character of disturbance of nervous activity should be closely defined in order to avoid the confusion that

has arisen and will still occur if traumatic concussion is not clearly differentiated from other kinds of disturbance of nervous reactivity. In some respects it is necessary to keep the different kinds of mechanism of production clearly identified.

With the rapid increase in knowledge of the condition in recent years it is a question whether a more accurate definition of cerebral concussion can now be devised. As the condition is primarily of clinical interest it is unlikely that any definition not based on the disturbance of consciousness or awareness would be generally acceptable. Though the primary event may be an excitation of nervous tissue, it is necessary to emphasize the transitory loss of nervous function which is the essential characteristic. Not everyone who "sees stars" is concussed. A physiological definition should also take into account the occurrence of a similar and probably identical phenomenon in the spinal cord (51) and peripheral nerve (61). It now has to admit histological change but not vascular or hemorrhagic factors. There is as yet no certain indication that complete lysis of nerve cells can be a direct effect. With these considerations in mind we suggest that concussion can be defined as a transitory and reversible nervous reaction with *immediate* onset following physical stress of sufficient violence and brevity, and characterized by progressive recovery thereafter. In man, amnesia both retrograde and postgrade is its chief external sign, accompanied by a loss of reactivity which is its clearest feature in animals. The loss of activity affects all tissue subjected to such stress but the duration of paralysis varies with the complexity of the nervous mechanism as well as the intensity of injury.

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# THE APPRAISAL OF NUTRITIONAL STATUS (NUTRITURE)<sup>1</sup> IN HUMANS

WITH ESPECIAL REFERENCE TO VITAMIN DEFICIENCY DISEASES

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Forty years ago Chittenden began the preface of his book *Physiological Economy in Nutrition* with the following words "There is no subject of greater physiological importance, or of greater moment for the welfare of the human race, than the subject of nutrition. How best to maintain the body in a condition of health and strength, how to establish the highest degree of efficiency, both physical and mental, with the least expenditure of energy, are questions in nutrition that every enlightened person should know something of, and yet even the expert physiologist today is in an uncertain frame of mind as to what constitutes a proper dietary for different conditions of life and different degrees of activity. We hear on all sides widely divergent views regarding the needs of the body, as to the extent and character of the food requirements, contradictory statements as to the relative merits of animal and vegetable foods, indeed, there is great lack of agreement regarding many of the fundamental questions that constantly arise in any consideration of the nutrition of the human body. Especially is this true regarding the so-called dietary standards, or the food requirements of the healthy adult. Certain general standards have been more or less widely adopted, but a careful scrutiny of the conditions under which the data were collected leads to the conclusion that the standards in question have a very uncertain value, especially as we see many instances of people living, apparently in good physical condition, under a regime not at all in harmony with the existing standards."

This statement was made four decades ago with special reference to the proteins. Today it applies very aptly to the vitamins. Much evidence has been collected in widespread efforts to determine the nutritional status of individuals and populations with respect to the vitamins. In the interpretation of such evidence much confusion has arisen, and from a given mass of observational data different workers frequently draw quite opposite conclusions. In this paper an attempt will be made to identify the causes of such disagreement.

One of the causes of confusion has been the use of ill-defined, and even paradoxical words (such as "sub-clinical" in describing disease). For the present discussion it is therefore desirable to define the chief terms to be employed.

*Nutriture* means "condition as to nourishment." It may be considered relative to one nutrient at a time, as is done below by the unqualified word, or relative to all nutrients, when it may be termed "general nutriture."

<sup>1</sup> Dr H. M. Sinclair of the Oxford Nutrition Survey has revived the use of this obsolete word. The definition quoted above appears in the Oxford English Dictionary, and makes the word a useful one for present purposes.

*Deficiency disease* means any departure from health due to lack in the body of some essential nutrient present in foodstuffs. Deficiency disease can be due to one of two causes (a) a *dietary deficiency*, i.e., use of a diet which contains an insufficient quantity of one or more essential nutrients, (b) an increased requirement above the usual level for one or more nutrients, due to what has been termed a 'conditioning factor' (1, 2, 3)

*Dietary standard* means a statement of the amount of one or more nutrients which must be ingested in order to attain a given result (which always should be, but frequently is not, clearly specified)

*Zones of nutriture* There are variations of nutriture from ideal to very bad, for this discussion we shall define five zones or levels of nutriture as follows

1 *Saturation* This is the state in which the body is incapable of increasing its content of a given nutrient even with prolonged ingestion of very large amounts. Presumably this should be nutriture at the highest possible level, except when the large amounts of the nutrient can work harm in the body. When this occurs one can speak of a zone of excess (e.g., hypervitaminosis A or D, obesity, or ketosis due to an excessive ingestion of fat)

2 *Unsaturated, but functionally unimpaired* This level of nutriture signifies that the body contains less of the given nutrient than at saturation, but that no clinically manifest or potential deficiency disease exists, and no known biochemical or physiological test indicates any functional abnormality

3 *Potential deficiency disease* When the departure from saturation is sufficiently great and sufficiently prolonged, the next lower level of nutriture is reached, in which potential deficiency disease is present. Such potential disease is regarded as existing when in the absence of clinical evidence of a deficiency (a) a new stress on the organism will cause a rapid development of the clinically manifest disease or (b) a suitable physiological or biochemical test yields evidence of decreased reserve functional capacity. Reserve functional capacity is understood to be the ability of the organism to withstand a stress without deviation from its usual physiological course

4 *Latent deficiency disease* This is the mildest clinically detectable form of deficiency disease. It is characterized by vague, indefinite, non-specific symptoms. Hess' statement (4) regarding latent scurvy is applicable to all such states: "The diagnosis of latent scurvy is based mainly on the reaction to specific therapy and on marked improvement when orange juice, potato or other anti-scorbutic food is taken. The symptoms themselves are suggestive but do not permit a definite diagnosis."

5 *Clinically manifest deficiency disease* Such disease may be mild or severe. Mild manifest deficiency disease is that in which the classical syndrome has not appeared but in which the symptoms and signs are highly suggestive, although laboratory tests and therapeutic trial may be necessary to establish the diagnosis. Severe deficiency disease gives rise to the classical syndrome and can usually be diagnosed without recourse to laboratory tests or therapeutic trial

CONCEPTS OF THE DEVELOPMENT OF DEFICIENCY DISEASES A widely held concept is that a deficiency disease develops in a series of stages from a decreased

concentration of the vitamin in the blood, followed by diminished body stores and excretion, to a functional impairment, to microscopic and finally to gross anatomical changes. This is a rational hypothesis, used by a number of investigators as a starting point for further enquiry. The sequence of events is not necessarily the same for each vitamin, but some such sequence may be expected to occur, during which the nutriture changes from a condition of saturation, to unsaturation, to potential deficiency disease, to latent deficiency disease, and finally to manifest deficiency disease. This simple hypothesis has been extended by Kruse (5) to cover distinctions which he makes between acute and chronic deficiency diseases. The extended hypothesis is based upon data obtained by gross and biomicroscopic examination of certain surface tissues (conjunctiva, cornea, lingual papillae, and gingivae) which are assumed to be specific "indicators" of the nutriture with respect to four of the vitamins. This assumption is untenable in view of the weight of evidence against it (cited below, page 335), consequently the extended hypothesis based upon such observations is unacceptable until positive evidence supporting it is presented.

A deduction frequently made in the past is that for every manifest case of deficiency disease caused by severe dietary deficiency, many persons must consume diets deviating less widely from completeness and therefore be suffering either from mild, or latent, or potential deficiency disease. Many attempts have therefore been made (a) to refine the methods of clinical diagnosis of mild and latent deficiency disease, (b) to devise biochemical or physiological tests which will detect potential deficiency disease. Unfortunately these attempts have met with little success, at the present time there are few, if any, clinical criteria or functional tests which are widely agreed upon as reliable and specific for the detection of latent or potential deficiency diseases.

An interesting suggestion has been made by Carleen *et al* (8) who postulated that for those water-soluble vitamins which enter into the molecular structure of coenzymes, the curve relating maximal rate of action of the enzyme to tissue concentration of the vitamin will be the well-known curve of dissociation of any enzyme. Assuming that failure of the enzyme to function rapidly enough is the underlying cause of a deficiency disease, one can then divide the curve arbitrarily into segments which correspond to the five zones of nutriture defined above. A slight dietary deficiency would cause the nutriture of an individual to sink perhaps to the third or fourth zone, and then remain unchanged. A more severe dietary deficiency might cause the development of manifest clinical deficiency in one of two sequences. First, the nutriture may deteriorate steadily, passing slowly down through the second, third and fourth zones, until the fifth is reached, second, the nutriture may change relatively slowly for a considerable time, and then plunge rapidly downward through the third and fourth zones into the fifth. There exists at present no evidence which enables us to choose decisively between these two views, in fact either sequence may occur, depending upon the conditions, or one may be characteristic of a deficiency of one vitamin, the other of deficiency of another vitamin. The first course would be compatible with the oft-quoted estimates (9, 10) of a high ratio of potential and latent deficiency diseases to mani-

fest deficiency diseases. But attempts in recent years to demonstrate objectively the existence of widespread potential and latent deficiency disease have met with great difficulty. This suggests that the ratio may not be high and so may support the second view against the first.

Mitchell (7) has recently reviewed the question of the ability of the organism to adapt itself to low intakes of essential nutrients and has suggested that dietary deficiency if not too severe may lead first to the less severe deficiency diseases, followed by an adaptation to the lowered intakes after which the signs of deficiency disease disappear. Definite adaptation to low intakes (intakes which by comparison with some recent dietary standards would be termed deficient) of calories (11) calcium (12) or thiamine (13) appears to occur. This view has also been discussed by van Veen (14).

**MEANS OF ASSESSING NUTRITURE** Methods of assessing nutriture are (a) clinical examination, (b) demonstration of a biochemical or physiological lesion, and (c) estimation of the past dietary intake. When severe disease exists, the clinical examination is a sufficient tool. Attempts are constantly being made to refine this tool, so that it may become possible to detect mild and latent diseases with it, and with less reliance on other methods (15, 16, 17). For the detection of potential and latent disease reliance must be placed on therapeutic trial and on the detection of biochemical lesions.

Before such biochemical tests are considered established criteria of nutriture, their validity must be satisfactorily established by experiments on *human* subjects in whom unquestioned deficiency disease is produced, or in whom the spontaneously occurring disease is correlated with a low intake of the specific nutrient and with laboratory data indicating bodily lack of that nutrient. The disease must be curable by moderate amounts of the specific nutrient without other environmental changes. Such findings must be capable of reproduction by independent investigators.

The estimation of the dietary intake of the essential nutrients for the assessment of nutriture is of secondary value at present. For patients with manifest deficiency disease there is likely to be an obvious correlation with lack of the specific nutrient implicated. The method is too unreliable for the detection of potential or latent disease, due to lack of suitable dietary standards with which comparison can be made and to lack of knowledge of factors modifying the requirements for the nutrients. These difficulties vitiate the argument for "prevalence of inadequate diets as shown by surveys"<sup>1</sup> stated in Bulletin 109 of the National Research Council on "Inadequate Diets and Nutritional Deficiencies in the United States" (18). Other criticisms of that argument are (a) The evidence obtained during depression years such as 1934 (19) yields no information about the dietary intakes of today. Most of the economically poorest classes of 1934 are relatively much better off now, and the consumption by them of better foods has risen sharply due to the improved economic condition and to educational campaigns. Ferguson and McHenry (22) have provided evidence of the influence of these factors over a two year period in their East York surveys. The

<sup>1</sup> i.e. Dietary surveys.

morbidity statistics for pellagra are of interest in this connection. In the eleven years 1933-1944 the number of reported cases of pellagra in North Carolina (20) was successively 833, 543, 732, 821, 576, 816, 214, 97, 60, 20, 15. Similar decreases have been observed in other Southern States (21). (b) Some of the data on which the argument is based are quite unreliable. For example the apparent very low intakes of vitamin C reported in Wayne County, North Carolina (18, tables 8-11), are much below the true intake, because when the estimates were made lack of knowledge of the vitamin C content of vegetables after the prolonged cooking generally used in this section led to the practice of counting zero ascorbic acid in all cooked vegetables. It has recently been shown (23) that this practice is quite incorrect, and that in fact much of the ascorbic acid ingested by the population studied does come from cooked vegetables. Therefore the earlier reported figures cannot be accepted by any critical reader as evidence of the true vitamin C intake of the population studied.

The results of determination of dietary intake may perhaps in the future be the indication for testing for potential deficiency disease just as today they are useful as supporting evidence for a diagnosis of clinically manifest disease, but not until the effect of given levels of intake of each nutrient has been observed in a suitable number of human subjects who are carefully watched for the development of clinical signs and symptoms. In view of the effect of various dietary patterns and of other environmental conditions on the requirement for each factor, it will be necessary to have a series of experiments to explore these influences, and to take account of them in diagnosis.

**PREVALENCE OF SUB-NUTRITION** With the given definitions in mind, a discussion of some of the evidence which has been collected can now be profitably attempted. Evidence concerning each of the five zones of nutriture defined above will be given, in order.

1 *Saturation* It appears to be widely recognized that few American subjects are saturated with any of the vitamins (18), nevertheless, some authors seek to define normality in terms of saturation alone, and imply that unsaturation unaccompanied by other signs represents a departure from normal (24). (This is discussed at greater length below.) From the incidence of obesity (25, 28) it appears that a fairly high percentage of the population is in the zone of excess nutriture as regards caloric intake.

2 *Unsaturated but functionally unimpaired* It seems possible that the majority of the American population falls into this group, with regard to one or more of the vitamins. Certainly it has been demonstrated in many studies of apparently normal subjects showing no signs of disease (18), that many<sup>3</sup> are unsaturated with respect to vitamin C and complete absence of vitamin C from the plasma has been observed in a considerable number of subjects (26, 27, 28) in populations where scurvy among adults is unknown. It is to be recalled that in a case of experimentally produced avitaminosis C, the human subject showed departure

<sup>3</sup> Where particular cases are cited in support of an argument, it is important to remember that the condition described may or may not apply to vitamins other than that mentioned. Each will require separate investigation to establish the point.

from saturation (as measured by plasma levels of ascorbic acid) within a week of beginning to ingest a scorbutic diet, while the first evidence of derangement of function was not detected until after 3 months' consumption of this totally deficient diet (29). In spite of this, there has been a tendency to define scurvy in terms of lack of saturation with vitamin C, thus Abt and Farmer (30) speak of scurvy co-existing with plasma levels up to 0.5 mgm per cent, and refer to a level between 0.5 and 0.6 mgm per cent as indicating "beginning scurvy". Certainly these levels may represent unsaturation, but they are very frequently met with among quite healthy persons and there is reason to doubt whether scurvy or any sign of avitaminosis C appears at all while there is any vitamin C in the fasting plasma.

In this connection the findings of Levine, Gordon and Marples (31) are also instructive. In apparently healthy male premature infants they observed the excretion of *p*-hydroxyphenyl lactic and *p*-hydroxyphenyl pyruvic acids in the urine and the disappearance of these metabolites from the urine following the administration of ascorbic acid. The excretion of these compounds constitutes a biochemical lesion, and *this lesion could be eradicated by administration of amounts of ascorbic acid insufficient to raise the fasting plasma level of ascorbic acid above zero*. It is to be noted that these children did not exhibit clinical evidence of scurvy. In assessing observations reported in the literature it is essential to note what criteria were employed for the diagnosis of scurvy. (This caution should be generalized to cover all deficiency syndromes due to lack of other vitamins.)

**3 Potential deficiency disease** The idea of potential deficiency disease is perhaps clarified by mention of examples. Among these we include the infants studied by Levine *et al* who excreted abnormal end products of metabolism of tyrosine and phenylalanine, individuals who are free from clinical signs of thiamine deficiency, but who have a decreased ability to clear pyruvic and lactic acids from the blood following exercise or the administration of glucose, and such individuals as those in whom Follis *et al* (32) found histological evidence of rickets at autopsy, although they may not have shown any detectable signs of rickets during life.

It is impossible at the moment to obtain any reliable direct estimate of the prevalence of potential deficiency states among the general population. The low incidence of clinically manifest deficiency diseases within the population leads us to infer that the reservoir of potential cases must be fairly small in number, or else the precipitating factors which determine the advent of the manifest diseases are almost totally absent.

**4 Latent deficiency disease** The diagnosis of latent deficiency disease, as emphasized above, rests upon the presence of *non specific symptoms*, upon knowledge of a probably inadequate intake, and upon a therapeutic trial. Laboratory data may be confirmatory. The symptoms are frequently those complained of by the maladjusted or neurasthenic patient. Ruffin (15) has pointed out that "vitamins afford little relief in social, financial or domestic problems." There exists a real dearth of reliable information about the prevalence of latent deficiencies,

because *all* the criteria mentioned above have not been applied to well-controlled groups

5 *Manifest deficiency disease* Mild deficiency disease has frequently been diagnosed from the presence of such signs as conjunctival changes, angular stomatitis, or changes in the appearance of the tongue. Such signs are not pathognomonic of specific deficiencies and may appear as a result of other causes (*vide infra*). At the present day there is very little severe deficiency disease encountered in this country, and the only one of the deficiency diseases to reach epidemic proportions in recent times is pellagra. Although morbidity and mortality statistics often are misleading, one can at least conclude from them that this disease is now quite uncommon. Experience of trained observers at such centers as Duke Hospital and Vanderbilt University Hospital show that it is now difficult to obtain even a few cases for study. The prevalence of mild manifest deficiency diseases is a matter of great doubt, since the diagnostic criteria are not generally agreed upon. From this it might be inferred that such diseases are by no means common, or they would have been recognized.

Before leaving the question of the prevalence of sub-nutrition, one reason for widely varying estimates of the incidence of deficiency diseases must be mentioned, this is the difference between the samples of the population encountered by different observers. In conducting a nutrition survey the investigator should either see the whole population of the chosen region, or a statistically representative sample of it. The investigator who follows either of these courses is likely to record a relatively small incidence of deficiency disease. On the other hand, the physician in a hospital out-patient clinic within the same region sees a highly selected sample which is not at all representative of the whole population. Such a sample is biased for its economic class and also for its consciousness of illness, and will present a relatively high incidence of deficiency diseases. This may well be the true incidence in the sample observed, but not of course *in the population at large*. The same criticism applies to those surveys which are carried out on selected economic groups, one cannot generalize from the results of surveys of such groups to the population at large.

**DIETARY STANDARDS** A dietary standard is a statement of the amounts of one or more nutrients which should be ingested in order to attain a specified result. As Leitch (33) has pointed out, dietary standards have undergone evolution. They have indeed evolved from early ones designed only to supply sufficient aliment to keep body and soul together, to some present-day standards designed to realize to the limit man's material capacity for life, and perhaps even to improve his moral condition as well (34). Those most in use at the present day are generally designed either as minimal standards to supply sufficient of the vitamins to ward off all signs of deficiency disease (35), or as so-called optimal standards, intended to maintain health at a higher level than a minimum standard can do (36).

It is not yet certain whether this distinction between good nutrition and optimum is valid. Many workers in the field accept the distinction, but others consider that there is not yet good evidence to establish its existence (37, 38). It is instructive to consider it in terms of nutriture. The minimum standard must be

reached in order to attain the second zone of nutriture defined above, and the "optimum" standard would then raise the nutriture to the first zone. The first question to be decided is, does saturation produce a degree of health and resistance to disease greater than unsaturation? There exist no satisfactory data to answer this question, and there is wide divergence of opinion about the answer which will ultimately be obtained. We incline to the opinion that beyond an intake which will prevent potential deficiency disease, further increases in quantity of water-soluble vitamins ingested will confer no additional benefits upon the organism, except perhaps the ability to survive longer periods of deprivation. Even this benefit is not always realized, as was clearly demonstrated for vitamin C in the guinea pig by Zilva (117). For the fat-soluble vitamins this is not true, since such massive storage of these factors can occur. When in the course of experimental work an improvement in the condition of man or animal is seen as a result of additions to the diet, it is to be concluded that before the addition was made, the diet did not reach a minimum standard for the prevention of potential deficiency disease.

A most important point which is not always kept in mind regarding dietary standards is that they give a single figure (comparable to a mean value) as a measure of a quantity varying over a range even for individuals of the same sex, height, weight, age and activity. *Since the dietary standard possesses the properties of a mean, its usefulness as a yardstick for assessment of the mean intake of a nutrient by a population is considerably greater than its usefulness when applied to evaluation of the intake of an individual.* Ideally the standard should be based on experimental determination of the requirement of the nutrient for maintaining the individual in that zone of nutriture which the standard is designed to attain. In that case, it will be calculated from the mean of the observed values and will be accompanied by an appropriate standard deviation for the group, which can be used to evaluate the variations from the mean in any population whose intake is compared with the standard.

It must also be remembered that dietary standards for the various essential nutrients are to some extent interdependent, and therefore hold for only a single dietary pattern. Thus the standards for the vitamins B are now generally set relative to the caloric intake. There is evidence (25) that the Recommended Dietary Allowance (36) of calories is too high, and it is therefore probable that the figures for the vitamins B should be scaled down accordingly. The requirements for thiamine also depend upon the fat content of the diet (39), these have been discussed recently by McHenry (40) who has concluded that "the allowance for thiamine now commonly recommended is much too large and that because of the use of this excessive allowance as a dietary standard, a great deal of the alleged deficiency is non-existent." Holt (41) has also recently concluded that the thiamine requirement on a diet chosen naturally lies between 0.17 and 0.23 mgm per 1000 calories. (This would mean about 0.5 mgm thiamine daily for a sedentary man.)

There is unfortunately a tendency to estimate the incidence of *deficiency diseases* by applying a dietary standard to the evaluation of measures of food intake



by the population at large. These estimates are likely to be very misleading, even when the intakes are accurately known, if they are interpreted with reference to a standard such as that of the National Research Council which is aimed at maintaining an "optimal" state of nutrition. Although such an interpretation is unwarranted and is not in keeping with the original purpose of the Recommended Dietary Allowances, much of the evidence for the existence of widespread potential and latent deficiency disease in the United States has been obtained in this way (18).

Another unfortunate circumstance is the very small amount of evidence upon which most of the dietary standards are based. Few human beings have ever been maintained on a deficient diet with precisely controlled quantities of one or more nutrients for a sufficient length of time to establish the intake levels at which deficiency diseases develop. Recent investigations suggest the possibility that the biosynthesis of thiamine (42), riboflavin (43) and nicotinic acid (44) occurs in some humans and therefore the minimal dietary requirement must vary greatly from one individual to another depending upon whether such "refection" is occurring and to what extent. With some of the vitamins, standards have been estimated in the complete absence of measurements on human beings, using as a basis only fragmentary data on other species (45). As a result, the tendency has been to make a high estimate of the actual mean requirement to prevent deficiency disease, then to make an extra allowance as a safety factor, and a further allowance to convert the "safe" minimal requirement into an "optimal" allowance. In such ways standards are devised which are very likely a good deal too high, the National Research Council standards can be criticised on these grounds.

Even for calories, about which we know far more than for any of the vitamins, and of which either an excess or a deficiency is harmful, there are suggestions that the figures may be too high. For instance, Milam and Anderson (77) consider 2000 calories the "general level of adult caloric intake" in a North Carolina population, and Youmans *et al* (25) have reported similar findings. Winters and Leslie (46), by actual analysis of duplicate meals found that among 24 women of a low-income group in Austin, Texas, the average intake was only 1145 calories per day. It might seem desirable to judge the adequacy of caloric intake of an individual by his weight and its variations, and to restrict the use of *dietary standards for calories* to evaluation of mean intakes of populations.

**THE CONCEPT OF NORMALITY** Ivy (47) has pointed out four different ways in which the word normal is applied to the structure, function and physico-chemical characteristics of the human body. One meaning, the "normative," requires that a normal person be one who is perfect according to some fixed set of standards. All others are regarded as abnormal (in nutritional condition they are the deficient). Such a meaning is used by Kruse in his discussions of nutritional problems, but as stated by Ivy "This view is obviously arbitrary and the authoritativeness of its standards may in many instances be questioned." This appears to be particularly true in nutrition work.

The second ("clinical") meaning defined by Ivy "maintains that anyone is normal who is well and not handicapped by some disturbance manifested by

symptoms. It views the mild imperfections of the body as normal." The remaining meanings are statistical "arbitrary statistical," which involves observation of a homogeneous population and definition of that portion of the population as abnormal which deviates from the mean by more than a specified amount, and "non arbitrary statistical," which "acknowledges no predetermined judgment and no arbitrariness." This last view maintains that "a sharp distinction between normal and abnormal does not exist for a group or even an individual."

It recognizes that degrees of normality and abnormality exist." It is likely that the clinical or the non arbitrary statistical meaning of the word normal will be the most fruitful to choose for application to nutrition work, both in order to advance our knowledge, and as a basis for the solution of practical nutrition problems.

A slight departure of bodily structure or function from ideal, not causing illness or curtailment of the activities, work or happiness of an individual, and only detectable by histological examination either during life (e.g., biomicroscopic examination of surface organs) or post mortem, should not be regarded as a *significant* abnormality. Indeed it might be argued that some such departures indicate the adaptation of the individual. It follows that the claims for startlingly high incidences of deficiency diseases diagnosed on the basis of such departures from the ideal are to be heavily discounted. For instance, histological studies of bone at autopsy have been claimed to show that 46 per cent of a group of 450 children dying in hospitals had suffered from rickets (32), biomicroscopic examination of a group of 49 people was said to reveal that all showed lesions characteristic of nicotinic acid deficiency and also lesions characteristic of ascorbic acid deficiency (48, 49). Comparison may be made with the presence of simple lentigo. If one chooses the non freckled individual as normal, all persons with lentigo would be termed abnormal. No one however would claim that such a condition would significantly affect the health of the individual. One cannot merely say "normal" or "abnormal," but must define the abnormality in terms of some measurable sign and demonstrate the significance of such abnormality.

The hemoglobin level affords a simple example from laboratory measurements pertinent to this discussion. It is frequently stated that the normal value for an adult female is 14 grams per 100 cc of blood. This is a normative view. It implies that a subject with any lower value is abnormal and classes her as anemic. It classes as anemic the woman with a hemoglobin of 12 grams per cent who judges herself, and is judged by her physician, to be in excellent health, and yet there are such women, whose hemoglobin cannot be raised permanently, or sometimes even temporarily, to the level of 14 grams per cent by therapeutic measures (50). Such women should not be labelled anemic or abnormal, and their existence brands the normative concept as ill-considered in its generalized application to health problems.

THE QUESTION OF SPECIFICITY IN DEPARTURES FROM NORMAL MORPHOLOGY SEEN IN SOME DEFICIENCY DISEASES. The question of specificity is acutely raised by the use of such single slight deviations from ideal, unaccompanied by any other signs, as diagnostic of deficiency diseases when such deviations are found in large

proportions of the population That is, are such deviations caused by the nutritional lack which is diagnosed, *and by that alone?* (In Ivy's terms, is the normative standard which has been employed authoritative?) Kruse has maintained that the changes resulting from a deficiency are tissue-specific Thus, he characterizes aniacinosis by tongue changes (48), avitaminosis A by conjunctival changes (51), avitaminosis C by gingivitis (49), and riboflavin deficiency by corneal invasion (52) A review of the published reports of other investigators fails to substantiate such a claim Thus, experimental gingivitis has resulted from a deficiency of vitamin A (53, 54), of vitamin D (55, 56), of vitamin M (57, 58, 59), of nicotinic acid (54), as well as ascorbic acid Furthermore, one may find scurvy without gingivitis (29, 102, p 89), gingivitis may develop after other signs of the disease (102, p 177), and, finally, gingivitis as it commonly occurs in an otherwise healthy group of individuals may not respond to vitamin C therapy (60) Jeghers (61) has reviewed the multitude of deficiencies and other diseases which may produce abnormalities of the tongue and has wisely cautioned that "Pellagra glossitis or ariboflavinotic tongue should not be diagnosed unless there is other supporting evidence for these specific deficiencies, or unless the tongue is unusually characteristic "

While it is well established that some cases displaying Bitot's spots yield rapidly to therapy with vitamin A (62), other cases are resistant to treatment with this vitamin (63), and may exhibit plasma vitamin A levels within the range which is usually encountered in healthy individuals (79) Berliner (64) has questioned the diagnosis of xerosis conjunctivae as depicted by Kruse (51) as evidence of vitamin A deficiency Darby and Milam (28) have found that the incidence of conjunctival changes described by Kruse was equally great in a population with a calculated intake of vitamin A of more than 2500 I U daily as it was in a population with a daily recorded intake of less than 2500 I U Finally, Kirkpatrick (65) noted in 1922 that night blindness was the first premonitory sign in what has now been established as clinical vitamin A deficiency Keratitis and corneal invasion may unquestionably be produced in rats by riboflavin deficiency (66, 67, 68), and some of the spontaneously occurring vascularity of the cornea in humans yields to riboflavin therapy (69) Similar changes, however, have been observed in the following deficiencies vitamin A (70), tryptophane or lysine (71), zinc (72), sodium (73), and some unknown factors (74, 75) Likewise, corneal invasion occurs in various diseases of the eye such as trachoma It has not been possible to correlate the dietary intake of riboflavin with the occurrence of corneal vascularity (28, 76, 77), and therapeutic response to riboflavin in many instances has not been successful (78, 79) It must be concluded, therefore, that there exists no specific tissue alteration which alone may be considered pathognomonic of a single deficiency state Furthermore, the basic question of what constitutes a departure from normality in the tissues examined by Kruse in his attempts to diagnose deficiency diseases is by no means a closed one

THE POSSIBILITY OF OPTIMUM NUTRITURE The use of the term optimum requires careful consideration, as it has generally been used without any clearly de-

defined meaning. The definition of optimum health is difficult, and the definition of optimum nutriture is perhaps still more difficult. How is optimum nutriture to be recognized? By size of the adult? By rate of growth? Not alone, for "a child may be destined to be tall or short, to progress rapidly or slowly through physiological time" (80). Furthermore, quantity does not insure quality, and there are disadvantages to the race in increasing the size of our bodies very far (81). By length of life? Only if we can measure its quality. Increased length of life, seemingly, may be promoted by more abundant intake of some factors, such as calcium (82, 83, 84, 85) or by a limited caloric intake (86). By resistance to infection? Here one must specify the type of infection, for it appears that while good nutriture may aid in warding off the effects of some bacterial agents (68, 69, 87) poor nutriture is a positive help in repulsing certain virus infections (88, 89, 90, 91). Aycock and Lutman (37) have recently reviewed the rôle which nutrition plays in warding off infection and have concluded "that vitamin deficiency as a factor in susceptibility to infection is not a general epidemiologic principle. The indications are that only deficiencies of certain vitamins affect susceptibility to certain types of infections and that these occur only in limited areas where these vitamin deficiencies reach a sufficiently severe degree to produce tissue changes which are favorable sites for secondary infection."

The term *optimum* has frequently been employed as synonymous with *maximum* when applied to nutriture. It is evident to all that such a concept is totally unacceptable as regards caloric intake. Thus obesity, which in all cases must result from a caloric intake in excess of the requirements of the individual (92), is associated with increased morbidity (93, 94) and increased death rate (95). Chittenden (96) expressed his concept of optimum nutrition as follows: "It is self-evident that the smallest amount of food that will serve to keep the body in a state of high efficiency is physiologically the most economical, and hence, the best adapted for the needs of the organism. Any excess over and above what is really needed is not only uneconomical, but may be directly injurious." Nicholls and Nimalasuriya (12) expressed a similar view: "A sufficiency of any constituent in the diet of healthy individuals is the optimum, nothing is gained by an excess, and as a general principle, dietary excesses should be avoided."

It appears that the term *optimum* has been used with a multitude of meanings, and that the meaning may vary for almost every user. It may well be argued that such a term should be expunged from the literature, and it does not appear to the writers to be one which can at present be of service in the advancement of nutritional science. A more valuable concept seems to be the definition of the two highest zones of nutriture which we have defined above as adequate or good, and the abandonment for the present of any distinction between optimal and adequate nutrition until a satisfactory meaning can be given to optimal.

The whole question of optimum as distinct from normal or adequate nutriture was most clearly discussed by Mitchell and Hamilton 15 years ago with respect to protein intake, and their conclusions may apply equally well to the intake of the various vitamins. They wrote (38, pp 569-571)

"Evidently the postulate of an optimum nutrition superior to merely adequate

nutrition cannot be substantiated until a most searching and successful study of nutritive requirements has been completed, in order to prove that the condition of adequate nutrition with which the optimum is to be compared is in truth adequate. It may seem that the whole question at issue here is merely one of the definition of terms, in particular the substitution of the word "adequate" to cover what is frequently referred to as "optimal." This substitution, however, replaces an indefinite term, appearing to involve implications difficult of acceptance, by a term perfectly definite in its significance. The prevailing dietary habits in this country, for no obvious reason at all, are considered merely adequate, hence the possibility of increased human efficiency and happiness following super-adequate nutrition.

"The great significance attached by Sherman to the marked improvement in the nutrition of rats induced by increasing the proportion of milk in the diet, is greatly diminished when it is realized that the inferior ration, containing the smaller proportion of milk solids, cannot fairly be called nutritively adequate, except insofar as it is capable of promoting *continuously* the different functions of animal life. However, the extent to which these functions operate should also be considered in assessing the adequacy of any dietary regime, i.e., adequate nutrition implies something more than continued health and existence. The experiments of Sherman simply afford an illustration of the different degrees of adequacy of different rations, and not of a super-adequate nutrition following upon increases in the consumption of milk."

If this point of view expressed by Mitchell and Hamilton had been carefully studied, much of the present day confusion and misconception about optimum nutrition need never have arisen.

**THE QUESTION OF SLIGHT CHRONIC DEFICIENCY DISEASES** A point requiring further investigation is the rate of disappearance of minor changes in surface tissues resulting from chronic slight deficiency in the diet. According to Kruse (5), such lesions require months or years to cure. As mentioned above, there are good reasons to doubt the specificity of the lesions studied by him. Furthermore, one of the most striking things ever noticed about severe deficiency diseases involving just those tissues which are the site of such changes is the rapidity with which cure and restoration of the tissues follows specific treatment with small amounts of the missing factor (15, 17, 62, 97, 98, 99, 100, 101, 102, p. 236). Yet it is claimed that large intakes of the appropriate factors will cure only a proportion of subjects of their slight chronic deficiency states in a year (48, 49, 51). The simplest conclusion from this is that the refractory conditions being treated are not specifically due to deficient intake, and that the tissue changes seen over such long periods (necessarily involving change in environmental conditions) are due to other causes than the addition of the one factor to the diet. Moreover Ruffin has observed (103) that when a chronic mild pellagrin has had a denuded red tongue for a long period, nicotinic acid therapy induces just as rapid a cure and regeneration of the papillae as occurs in the recently affected tongue of an acutely ill pellagrin when he receives similar treatment. Satisfactory evidence of widespread mild chronic deficiency diseases which can only be very slowly

cured has not yet been obtained, and it appears to be reasonable to doubt that it will be obtained in this country under existing economic circumstances

The claim has been made that a great deal of ill health and incapacitation due to chronic latent or mild deficiency diseases occurs in this country (104, 105), but again the evidence to establish the claim soundly is wanting. To cite once more the pellagrin, as seen in North Carolina and Tennessee, there is little doubt that the acutely sick pellagrin has often suffered from a chronic latent deficiency of nicotinic acid for a considerable time, and that he is only precipitated into the acutely sick stage by an additional trauma. Frequently the farmer becomes acutely ill after a long day's exposure to the intense sunlight of early summer while working in his fields (106). In other words, he carries on his usual activities through the chronic latent or mild stage, and is only incapacitated after the onset of the acute severe disease.

Exactly the same sort of thing has been reported by Platt and Lu (100) for one type of beri-beri seen in the Orient. The patients continued their ordinary activities without difficulty and then suddenly collapsed and without specific therapy died in a few hours. Until much more objective and precise evidence appears, there is no reason to doubt that the same holds true of deficiencies of the other vitamins.

Experimental evidence bearing on this point must be most carefully controlled because it is likely to be based on subjective symptoms and reactions of the subjects, who may be abnormally confined and restricted or overtaxed by the burden imposed during periods on test diets. The use of large numbers of subjects and really adequate controls will help to prevent erroneous conclusions from being drawn. Muench (107) has emphasized the need for particular care in design of experiments in this field, and has mentioned some criteria which are often neglected.

Marrack (108, pp 52-3) has epitomized the difficulty of interpretation of the evidence of slight deficiencies among the population at large—"With human beings it is not so easy to be sure about these minor deficiencies as they occur in those unplanned experiments on human beings in which the level of the diet is lowered by force of circumstances, economic or otherwise, or in response to taste or custom. Not only may the food be inadequate in several respects, but other unfavorable conditions, such as bad housing, may operate at the same time. The symptoms may be ill-defined, usually they may be attributed to several possible causes. Specialists in the appropriate branches of medicine would probably be able to incriminate a complex, a hormone defect or a surgical condition. People may get better without treatment or with any form of treatment. Before concluding that any *symptom* is due to deficiency of a particular vitamin, we should insist that the incidence of this symptom should be related to the amount of this vitamin in the patient's diet and that there should be a higher rate of recovery among patients treated with this vitamin than among control cases. The most satisfactory information is that obtained from the few deliberate experiments in which the vitamin supply of human beings has deliberately been limited."

**THE DIRECTION OF FUTURE ADVANCE** One of the advances which will greatly facilitate the detection of the potential and latent deficiency diseases is the refinement of existing biochemical and physiological tests, and the invention of new and more delicate ones. At the present time very few of these tests are really reliable for diagnosing a latent deficiency in an individual. The visual threshold measurement at complete dark adaptation is an example of a useful procedure of this sort. This gives the earliest hint of vitamin A deficiency, *but only if* a raised threshold is quickly lowered following specific therapy (109).

Some progress is being made with other tests. It may be claimed that several are already of real value in the detection of potential and latent deficiency diseases, although in general they are still too cumbersome for widespread application. Attempts to simplify such tests sufficiently to make them applicable to nutritional surveys of populations are already meeting with some success. Examples of such tests are the measurement of ascorbic acid in the white cell-platelet layer (110), the rate of disappearance of blood pyruvate following exercise, as a measure of thiamine deficiency (111), fasting urinary excretion of thiamine, riboflavin and nicotinic acid (112), and simplified tests for the excretion of thiamine, riboflavin, and nicotinic acid metabolites following the administration of a fixed dose (113). These can be used to demonstrate differences between the average responses of different groups, but are of much more equivocal value in estimating the nutriture of the individual. This arises from two causes, the variation between different individuals which occurs for all attributes of living organisms, and the difficulty in fixing a level of response below which with a stated probability a deficiency disease will co-exist. This latter difficulty will persist until such tests can be carried out at intervals on subjects maintained on deficient diets long enough for the severe deficiency disease to appear, or alternately in the "experiments of nature" in which the severe disease develops on the usual diet of the individual. (Such subjects are unavailable in sufficient numbers in this country today). Only then will it be possible to evaluate the results of the tests in terms of clinical condition. An interesting example of this is provided by attempts of investigators in the U.S.A. to fix a "lower limit of normal" for the plasma vitamin A. Estimates have varied from 75 I U to 130 I U per 100 cc (114). Recent work in China by Hsu (115) has demonstrated that patients with clinically manifest avitaminosis A had a mean level of 6 I U, while his normal controls had 54 I U, and hospital patients without clinical avitaminosis A had 36 I U. Thus it appears that in this country there had been a failure to set a level below which deficiency disease occurs, simply because of the rarity with which such disease had been encountered. This conclusion is strengthened by failure to find evidence of impaired dark adaptation in various districts (116). Almost everyone here appears to have a sufficient supply of vitamin A, so that no evidence could be obtained about plasma levels of deficient people.

It is well to bear in mind the distinction between three types of biochemical test: (a) measurement of vitamin levels in the blood or urine of the untreated subject, (b) similar measurements for the subject after oral or parenteral administration of the vitamin ("load" or "saturation" tests), (c) detection of metabolic

products in the blood or urine which do not usually appear except as a result of a deficiency (functional test) It is possible to have a low level of a vitamin in the blood, or a low excretion in the urine, and yet have no functional impairment (31) This is in accord with the findings of Zilva (117) in work with the guinea pig He demonstrated that urinary excretion of vitamin C occurs only after the tissues have been saturated with the vitamin It seems possible then that the measurement of urinary excretion of vitamin C by the untreated subject merely determines his intake in excess of that which can be utilized or destroyed by the tissues It is also possible to have a low level of vitamin C in the blood and yet to respond to a load test in the way a normal subject responds Kajdi *et al* (118) have reported one of the very few studies in which a load test has been correlated with presence or absence of manifest deficiency disease The essential point of their test was the measurement of plasma ascorbic acid 4 hours after intramuscular injection of 200 mgm of ascorbic acid, and the result was reported together with the observed signs for presence or absence of scurvy The conclusion was that 4 hours after the injection values below 0.2 mgm per cent occurred with clinical scurvy, and values above this level indicated absence of scurvy Unfortunately this test does not appear to have been followed up by any other workers in the field

For several years the sum of the evidence has suggested that fasting blood plasma levels and excretions of ascorbic acid following test doses have little relation to bodily requirement of the vitamin Such measurements locate the nearness of the body to saturation with the vitamin, which is probably irrelevant In a recent review Pijoan and Loxner (119) reach similar conclusions "low plasma values do not necessarily indicate a scorbutic process unless such values exist concomitantly with a deficiency of the white cell platelet content," and "a steady depletion or continued lowering of the plasma value and subsequently the white cell platelet layer indicates a dietary shortage, and when the white cell platelet layer levels become completely depleted scurvy follows"

Experience with the production of all grades of deficiency disease in human subjects will be necessary before the dietary standards can be made as satisfactory as possible Even then the standards will, as emphasized above, be chiefly of value in assessing the average intake of groups, it is to be expected that they can be improved in accuracy so far that one may be justified in diagnosing the presence of deficiency disease in a proportion of a population whose average intake is sufficiently far below the standard This cannot be done with the recent "optimal" standards which have been used so widely It must be realized that the standards for some vitamins were fixed in the absence of any observations on human beings on which to base them, as Sinclair has said, "our knowledge of these comes at present partly from guess-work and the guesses have not always been very inspired" (6)

There is a real need for two advances in the formulation of dietary standards In the first place, a dual standard should be drawn up, a higher standard to serve (as the Recommended Dietary Allowances of the National Research Council was intended to serve) as a "tentative goal toward which to aim in planning practical



dietaries" (36), and a lower standard representing the closest possible approximation to the average *requirement* for the maintenance of the individual in the second zone of nutriture defined above. This lower standard would then be one with which to compare dietaries in order to assess their *adequacy*, a given dietary could not be classed as *deficient* because it was below the higher standard, but could be so classed if it fell below the lower standard. If such dual standards were available, there would be much less tendency for the uncritical to claim that deficiency diseases are rife because the dietary intake fails to reach the higher standard. The second much-needed advance is to attach to the standard for each nutrient, (which has the properties of a mean) the appropriate measure of deviation in order that it may indicate the extent to which the standard requirement varies from one individual to another, as pointed out by Pett (120).

From what has been said it is clear that the widely held belief that general dietary improvement in this country will result in greatly decreased morbidity, increased productivity, more satisfactory personal adjustments and other evidence of "more positive health" remains a hypothesis. This may not be subject to direct test, but it would appear that the investigation of possible correlations of these qualities with the level of nutriture of well chosen samples may point the way toward further studies to explain the significance of any correlations which are found to exist. Such studies might be profitably undertaken within a population which has already been under careful observation.

The present widely held views regarding human nutrition, alleging the widespread occurrence of deficiency diseases in this country, have been based on uncritical acceptance of doubtful diagnostic criteria and on the improper application of tentative dietary standards. The confusion and contradictions mentioned in the opening paragraph have arisen from a failure of some workers in this field to use their critical faculties in digesting the available observational data and in devising experiments to test their hypotheses adequately. There is an urgent need for an author who, in the words of Funk, will write "with the intention of giving a summary of the modern investigations, and by means of a careful selection of references to facilitate the research for anybody who wishes to read the original literature. This careful selection (is) absolutely necessary, for there is perhaps no other subject in medicine where so many contradictory and inexact statements (are) made, which instead of advancing the research retarded it by leading investigators in a wrong direction" (121).

Further advance can only come through a great deal of carefully controlled work on human subjects. Progress in the last 10 years has been disappointing, largely because "fed by inadequate evidence a fire of uncritical enthusiasm to detect and treat imagined deficiencies has swept our countries, its flames fanned by certain political and vested interests" (6). Progress will not be possible until the present position is viewed clearly. Humiliating though it may be, it is necessary to realize how imperfect is our scientific knowledge of human nutrition for application to the conditions of men and of nations, before we can begin to extend that knowledge in an intelligent way. Meanwhile, in applying the knowledge we already possess, "We must base our demands on our positive knowledge,

not on the gaps in our knowledge" (108, p. 89) and we must firmly reject the palpable absurdities which are offered us. For the future we require a far more comprehensive account of the biochemistry and physiology of the vitamins before we can answer satisfactorily many of the problems which physicians and public health administrators pose today. Of one thing we may be reasonably sure, the recent tendency to find the answer to most public health problems in terms of nutrition will disappear. The introduction and universal consumption of an ideal diet would not ensure abounding health, it would lay only one of the foundation stones on which such health might be based. A multitude of environmental factors, including as not the least important the individual's reaction to his fellow members in human society, must be in correct adjustment before such health can ensue (122).

#### SUMMARY

A classification and definition of zones of nutriture (or degrees of nutritional status) is made as follows. Saturation (with sub zone of excess), unsaturated, but functionally unimpaired, potential deficiency disease, latent deficiency disease, clinically manifest deficiency disease. It is suggested that the second zone represents adequate or good nutriture, and that there is no cogent evidence that any higher level is more beneficial to health in the human, in this case, "optimal" nutriture can not be differentiated from adequate nutriture.

The data for assessing nutriture are of three types: those obtained by clinical examination, by biochemical and physiological tests, and by measurement and estimation of the food intake. Clinical examination detects only the well developed deficiency disease and must be supplemented by other evidence. Recent claims that the diagnosis of deficiency diseases of some of the vitamins may be made solely on the biomicroscopic examination of certain tissues have been weighed and found wanting. The interpretation of most of the biochemical tests is uncertain because these tests have not been correlated with the production and cure of the clinical signs of the disease in human subjects. The dietary standards which are widely employed in estimating dietary adequacy were not designed as standards of adequacy and are not founded on sufficient data on human subjects, consequently they give false excessive measures of the incidence of deficiency conditions. The estimates of widespread deficiency disease in this country (brought together in Bulletin 109 of the National Research Council) depend largely on estimation of the dietary intake and upon biochemical tests, and are therefore unreliable.

Future advances in the means of appraising the nutriture of the individual will rest upon elaboration of our knowledge of the biochemistry, physiology, and pathology of the vitamins in man. This can be attained 1, by investigations in which clinical deficiency diseases are produced and cured in a considerable number of subjects, 2, by careful studies of those spontaneously occurring cases of dietary deficiency disease and of conditioned deficiency disease. Such studies will lead to more accurate evaluation of the nutriture of populations, and facilitate the search for correlations between nutriture and the quality of health of a population.

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## THE PROTEINS OF PLANTS

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The existence of proteins in plant tissues was first recognized by the Italian natural philosopher and physician Beccari in 1728. His observations were not published until 1745 (1), but he demonstrated in a lecture given at the earlier date that wheat flour contains a substance which yields an alkaline liquor on dry distillation and which also putrefies, when exposed to the air in a warm moist place, in a manner indistinguishable from the behavior of what he termed "animal substance." These crude tests were adequate to show that the gluten prepared by washing wheat flour dough with water until the starch is removed is entirely different from the starch itself, and to permit its classification as a substance allied in composition with the flesh of animals.

During the last part of the eighteenth century and the first two decades of the nineteenth, such investigators as Rouelle (2), Fourcroy (3), Emhof (4), and Taddei (5), together with many others, greatly extended the knowledge of this and of other analogous products obtained from plants. To Fourcroy we owe the term albumin and the observation that plant saps contain substances allied in solubility and composition to the white of egg, to Einhof the observation that a part of the gluten of wheat flour is soluble in alcohol and that similar products can be prepared from rye and barley. He also noted that leguminous seeds contain albuminous substances different from those present in wheat. Taddei separated wheat gluten into two well differentiated parts by means of alcohol and designated the alcohol-soluble component gliadin.

The fact that these plant products contain nitrogen as well as carbon, hydrogen, sulfur, and oxygen was apparently first clearly recognized by Fourcroy, but the earliest comprehensive attempts to determine their composition were made by Boussingault (6) in 1836, by Mulder (7) in 1838, and by Liebig and his students (8, 9, 10) in 1841. Mulder stated his conviction that proteins are the "foodstuff of the entire animal kingdom and are probably synthesized only by the plants," an idea that was seized upon by Liebig and developed into a broad generalization which maintained that there are only four kinds of protein in nature, albumin, casein, fibrin, and gelatin. The animal was supposed to acquire these proteins from plants either directly in the case of graminivorous animals, or indirectly in the case of carnivorous animals. The part played by the animal was merely, to quote the striking phrase used by S. W. Johnson (11, 12) in 1867, that it "moulds over these vegetable principles into the fibrine, albumin and casein of its muscle and other tissues, of its blood, milk and other secretions."

Although greatly oversimplified, these views placed extraordinary emphasis upon the importance of plant proteins and, accordingly, it is rather surprising that so little attention was paid to these substances during the second half of the nineteenth century. Many observations of great significance were of course

made in this period by one or another of the leaders in chemistry and physiology, but only three investigators of great eminence chose to devote their whole attention to the fundamental problems of the chemistry of the proteins of plants. These were Ritthausen, Schulze, and Osborne.

Ritthausen (1826–1912) is remembered today as the investigator whose careful and painstaking studies of the proteins of plant seeds, extending over the period from 1862 until his retirement from the professorship at Königsberg in 1899, laid the foundation of our knowledge of these substances. His book "*Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen*" (13), published in 1872, stated many fundamental principles that must still be considered by all who work with seed proteins, and for the first time suggested that the determination of amino acids is the most satisfactory chemical method for the characterization of proteins, a suggestion that lay dormant for thirty years for want of suitable analytical techniques. In addition, Ritthausen discovered both glutamic (14) and aspartic (15) acids as products of the hydrolysis of proteins, and he was also able to show that Liebig's views regarding the limited number of proteins in nature required modification inasmuch as he encountered a number of seed proteins which could not be fitted into Liebig's highly simplified scheme. Nevertheless, Ritthausen was content to group together under the same designation all proteins from whatever source that corresponded with each other in their solubility relations and had approximately the same composition with respect to carbon, hydrogen, nitrogen, and sulfur. These criteria were, in fact, the only ones available to him for the characterization of proteins in spite of his belief that the determination of the amino acids produced by hydrolysis would furnish superior analytical evidence of difference or identity as the case might be. His work was indeed carried out at the dawn of amino acid analytical chemistry and no suitable quantitative methods were developed until Kossel (16) entered the field in 1898 and devised methods for the determination of the basic amino acids.

Schulze (1840–1912) is the first of the students of plant chemistry whose interest and accomplishments in the field of metabolism entitle him to be regarded as a biochemist in the modern sense of the term. His work was begun in 1872, and he concerned himself for many years with the chemical phenomena that occur when plant seeds sprout and develop into seedlings. One of the most conspicuous of these events is the disappearance of the reserve protein of the seed and the enrichment of the tissues of the young plant in asparagine, the amide of aspartic acid. Schulze showed, by analytical chemical methods, that there was insufficient aspartic acid in the proteins of the lupine seeds with which he worked to account for the very large quantities of asparagine that were formed, and accordingly he concluded that this substance arose as the result of a secondary process. It required many years of the most painstaking research to unravel the nature of this process, but in 1898 (17) he was in a position to state that, under normal conditions, the protein of the seed is hydrolyzed by proteolytic enzymes to amino acids, that these are oxidized with the production of ammonia, that the ammonia then combines with what he called nitro-

gen free residues, which were presumably derived from the carbohydrate metabolism, to form asparagine, and that the asparagine is transported into the growing parts of the plants where it serves as one of the most important sources of nitrogen for the synthesis of new protein in the developing tissues. This hypothesis of the metabolism of protein in seedlings has stood the test of subsequent investigation and remains, with minor elaboration, the basis of our present day views.

Schulze's direct contributions to protein chemistry are equally fundamental. He discovered phenylalanine in 1879 (18), and arginine in 1886 (19), both as products of the metabolism of proteins in lupine seedlings. Glutamine, the amide of glutamic acid, was first isolated from the water extract of beet-root tissue in 1883 (20). Schulze thus shares with Emil Fischer the unique distinction of having discovered no less than three of the amino acid components of the intact protein molecule.

Osborne (1859-1929), the third of the great nineteenth century plant protein chemists, did the greater part of his outstanding work on the isolation of the proteins of seeds during the last decade of the century. Because of his later investigations with Mendel upon the nutritive properties of proteins, which extended from 1910 until his retirement in 1928, Osborne's work is far more widely known than is that of the other two. Nevertheless it is apparent, from the more recent literature that deals with the preparation of seed proteins, that few present day students of these substances take the time to study Osborne's early papers with the attention they merit. References and credit are given, to be sure, but it is seldom that a full appreciation of what he really accomplished is indicated.

Osborne's work was done before the development of modern theories of the solubility of proteins made generalizations upon the effect of the salt concentration and hydrogen ion activity of the solvent possible, to say nothing of the effect of the dielectric constant. The concept of the isoelectric point, the use of buffer solutions, even the general acceptance of the view that proteins are ionizable electrolytes which can combine with acids and with bases, all came later, although on this last point Osborne was himself entirely clear. Yet in all of his early work, Osborne demonstrated a remarkable if instinctive appreciation of the fundamental relationship between the solubility of the protein and the salt content, the acidity, and the temperature of the solvent, and brought about his separations of the protein components of the extracts from seeds by well-conceived manipulations of these factors. For the isolation of the most soluble components, he was accustomed to dialyze the aqueous solution into alcohol, thereby altering the dielectric constant of the solvent and precipitating these substances in a form suitable for further study. In 1902, he published two papers on the basic character of the protein molecule (21) in which he showed that edestin, a typical seed globulin, enters into ionic reactions with acids to form true salts,<sup>1</sup> in 1905 he published a solubility curve of this same protein

<sup>1</sup> The first of these papers deals with the behavior in aqueous solution of a compound that edestin forms with acid which decomposes spontaneously to liberate the edestin in



(22) in salt solutions which showed both the ascending limb of the salting-in and the descending limb of the salting-out effects. These were pioneering attempts to formulate the principles of solubility upon which all of his protein isolation studies had been predicated.

During the early part of his work, Osborne was still more or less under the influence of the older ideas that assumed identity between proteins of different origin provided that they had the same solubility behavior and elementary composition. The advent at the turn of the century of the simple nitrogen distribution method of Hausmann (23) and of the powerful analytical methods of Kossel, enabled him to subject his preparations to more searching analysis with the result that he soon became convinced that there were few if any cases in which the same protein occurs in different plant species. He came to regard the demonstration of difference as being far more important than that of similarity and later, with the collaboration of Wells (24), was able to push these investigations to their logical conclusion by the application of immunological tests. The outcome was the generalization that, with two or three relatively unimportant exceptions in which the experiments were inconclusive, all known seed proteins are specific substances each being different in some respect from the others.

Up to the conclusion of this phase of Osborne's work, the concept plant protein had been more or less restricted to the fairly well known proteins of seeds. Schulze had given some attention to the metabolism of proteins in the growing green tissues, and agricultural chemists were, of course, concerned with the analytical determination as well as with the nutritive properties of the proteins of green fodder plants. Green leaf cells had long been recognized as being rich in proteins, but there had been few attempts to isolate these substances and very little was known of their properties. In 1920, however, Osborne and Wake-man (25, 26) in this country and Chibnall and Schryver (27) in England independently began their studies of the proteins of the green leaf which were subsequently continued for many years by Chibnall. Techniques were developed whereby samples of protein could be prepared from leaf tissues and subjected to chemical analysis. It was apparent from the first, however, that the products obtained, although reasonably pure with respect to gross contamination with carbohydrates or pigments, were far from homogeneous with respect to protein components, and they were invariably secured in a denatured condition with a solubility that differed profoundly from the native material characteristic of the living cell. Nevertheless, these preparations served admirably for the study of the nutritive properties of leaf protein and also for the investigation of the amino acid composition, although their properties could shed little light upon the chemical behavior of the protein systems of the living cells.

Chibnall's more recent investigations were thoroughly reviewed by him in his Silliman lectures delivered at Yale University in 1938 (28). In these he pointed

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an insoluble condition. He says, "This derivative of edestin is formed by hydrolysis, the amount formed being proportional to time and the concentration of the solution in hydrogen ions," a statement that has a thoroughly modern ring.

out clearly that the preparations of leaf proteins with which he had been concerned were undoubtedly mixtures of many components of more or less similar solubility relations, the only discrimination possible with existent techniques was that between the protein associated with chlorophyll and other pigments and lipids in the chloroplasts and the colorless and almost lipid free material derived from the cytoplasm of the cell

The concept plant protein has thus been broadened in the last quarter century to include not only the seed proteins but also an entirely new class of products. These substances have now become available, although usually in an altered and denatured form, but there is no reason to assume that improvements in methods will not ultimately make it possible to secure specimens in an unaltered form for study. In fact, as will be pointed out, some advance in this direction has already been made in the case of the chloroplastic proteins.

**PLANT PROTEINS** It may be well to consider in some detail just what is meant by the term plant protein. In the first place, the word protein is used with two fairly clearly distinguishable implications. The more restricted use applies it to specific substances which have frequently received designations and which, in the ideal case, have been demonstrated by chemical and physical tests to be homogeneous. Although it is doubtful that this ideal has been fully attained in more than a few, even if any, specific cases in the plant field, the statement that gliadin or zein or edestin is a protein is not ambiguous, or is at least no more so than the corresponding statement for the animal proteins such as ovalbumin or hemoglobin. The mental reservation that is made concerns the individual preparation of the substance. A given preparation probably contains a greater or less proportion of contaminating material, or may be a system of components that differ only slightly from each other. Nevertheless, however crude, it is an approach to an ideal, and it is to this ideal that the specific designation, or the general term protein belongs. We employ exactly the same restriction when we say that sucrose is a sugar. Here, the chances are that the sucrose, even if purchased at the grocery, contains only a small fraction of one per cent of contaminating material other than moisture. In the case of a protein preparation this percentage will doubtless be considerably larger.

The term protein is also employed in a more general sense as in the phrase seed protein or leaf protein. Here, the emphasis is upon the sum total of the substances in the seed or leaf which as individuals conform to the restricted definition of the word. In this sense, protein is a collective noun, and it is implied that a given preparation is a mixture of more or less related substances. The term purification as applied to material in this category refers to the removal of contaminants of non protein nature.

The classification of proteins is an old problem to which no adequate answer has even yet been given. It is fairly easy to discriminate between, for example, the alcohol-soluble prolamins of the cereal grains and the salt solution-soluble globulins of many kinds of dicotyledonous seeds. Solubility is and will probably remain for many years the basis of classification but the weaknesses of

this approach become more and more obvious as our knowledge broadens. An albumin is said to be soluble in water while a globulin is soluble only in the presence of salt. Yet the solubility curves of both albumins and globulins show a rising limb as the salt concentration is increased to a certain point, and a descending limb on further increase. It would be difficult indeed to draw a line on such a diagram and say that all proteins the solubility of which places them above this line shall be called albumins, all below it globulins.

The classification of plant proteins is rendered the more difficult by the fact that they occur in nature almost invariably in complex mixtures and thus, by interaction, modify each other's solubility. Accordingly as procedures for the isolation of individual components are carried out, the operator encounters what appear to be changes of the solubility. These sometimes may even lead him to entertain the suspicion that the solubility of the component in which he is interested has been altered during the experimental procedure, although this suspicion may, in fact, be groundless.

For practical purposes a rough classification of plant proteins on the grounds of location in the plants is, however, possible. This is the classification that has been tacitly used in the above discussion, and divides these substances into the proteins of the seeds, which serve primarily as the nitrogenous nutriment of the developing embryo, and the proteins of the physiologically active tissues of the leaves and stalks, and presumably also of the roots. The distinction bears many analogies to an attempt at a classification of animal proteins on the basis of whether they are derived from the hen or from the egg and has similar disadvantages. Nevertheless, just as a moderate degree of success attended early efforts to isolate and purify the chief proteins of the egg, so a considerable degree of success has been attained in the isolation and purification of many seed proteins. However, it is true that, with the conspicuous exception of animal proteins such as some of those found in milk or in blood, or in certain glands and specialized tissues, our knowledge of the individual proteins of the actively metabolizing cells either of the plant or of the animal is limited indeed.

**SEED PROTEINS** The higher flowering plants are divided botanically into two main groups according to, among other fundamental differences, the structure of the seeds. The dicotyledons produce seeds that more or less readily split into two halves, or cotyledons, located between which is the embryo which develops into the young plant when the seed is exposed to the proper conditions. The monocotyledons on the other hand, the group to which the cereal grains belong, bear seeds with only a single analogous storage organ usually called the endosperm, which is, in most of the cereals at least, characterized by the presence of a considerable amount of starch. The embryo is attached to the endosperm.

The cotyledons, or endosperm, are in all cases the locus of the essential food materials required by the embryo for its initial development, and this food is laid down chiefly in the form of the relatively stable substances, starch, protein and fat, together with a moderate proportion of inorganic material. The enzyme systems essential for the normal metabolic processes are likewise present,

and the entire structure is a living and respiring organism (29) in which life can persist often for many years provided that the seed is maintained in a dehydrated condition. When exposed to the proper conditions of moisture and temperature, the enzyme systems come into full action and the phenomena of growth are initiated.

Just as in the case of any other living organism, proteins of many different kinds are present, and it is therefore not surprising that there is scarcely a single species of plant seed the protein content of which can be accounted for at all adequately in chemical terms. The proteins of wheat have probably been studied more thoroughly and extensively than those of any other seed, yet the recent monographic treatment of the subject by C. H. Bailey (30) indicates that there is no general agreement among the large number of investigators who have concerned themselves with these proteins as to what they are or as to the chemical relations among them. Bailey concludes his chapter on the prolamins with the statement, "That various gluten protein preparations are not 'pure' in a strict chemical sense seems obvious. Whether these preparations are merely mixtures of a limited number of chemical entities, or are made up of a graduated and well nigh infinite number of components, extending regularly in their chemical and physical properties from one extreme to the other, is not fully apparent." His discussion of the glutelins indicates that even less that is clear and definite is known about this obviously complex group of components of the protein system, whereas only fragmentary information has been recorded concerning the water-soluble and salt solution-soluble proteins, the so-called albumins and globulins of the seed.

Dicotyledonous seeds in general contain as their main protein component one or more globulins which, in the case of many of the oil seeds such as the nuts, hempseed, tobacco seed and the seeds of the cucurbits, can under correct conditions be obtained in crystalline form. Although in itself no guarantee of homogeneity (31), the separation of crystals suggests that the solid phase is at worst a relatively simple mixture of closely allied components and that there is no gross contamination with non protein material. Most of the information in the literature of seed proteins refers to preparations of these main globulin fractions. They are assumed, and with a high degree of probability, to represent the chief storage protein of the cotyledons;<sup>2</sup> as a rule they appear to be relatively stable substances and can be subjected to repeated separation from solution or to recrystallization without conspicuous change in properties. Rigid tests of homogeneity have been only rarely applied to products of this type, however K. Bailey (34) has pointed out that even his most carefully prepared and purified samples of crystalline edestin, although homogeneous in the ultra

<sup>2</sup> Whether or not the so-called storage proteins share to any degree in the metabolism of the resting seed is unknown. However they are intra-cellular proteins and can be recognized by suitable microscopic technique as granules which in the case of certain oil seeds are indistinctly crystalline (32). During the period when the seed is reaching maturity and again when the process of sprouting has become established these proteins undoubtedly take an active part in the metabolism (33).

centrifuge, had a solubility that was to a large extent dependent upon the solute-solvent ratio either in polar or non-polar solvents. Fractions which differed from each other in gross solubility behavior could be obtained, but no fraction obeyed the ideal solubility laws. Accordingly even in this especially favorable and well-known case, chemical homogeneity cannot be claimed.

It is unfortunate that the powerful electrophoretic methods that have been so strikingly successful with many kinds of animal proteins are not in general applicable to the seed globulins. These substances are so insoluble at the low temperature and ionic strength conventionally used for such experiments that successful records of the moving boundaries cannot be obtained unless one is prepared to work at strongly alkaline reactions. Fontaine<sup>3</sup> has, however, secured evidence that three main electrophoretic components are present in the globulin fraction of the peanut. Two of these could be provisionally identified with the arachin and conarachin of Johns and Jones (35) but, inasmuch as the analysis was carried out at a pH somewhat greater than 9, the interpretation of the results is still tentative.

In addition to the main storage globulin or globulins which can usually be readily isolated, dicotyledonous seeds invariably contain other protein components. The literature provides little information regarding the relative proportions in which any of these substances are present. Analytical methods whereby different individual proteins can be distinguished are poorly developed and about all that is usually attempted is to record the relative proportions of the total nitrogen of the seed that are soluble in a series of solvents chosen so as to bring successively into solution the main classes of proteins that are expected to be present. Preparations that are presumed to represent these main groups are then isolated and subjected to more detailed analysis, but it is obvious that, with the possible exception of the major storage protein, there is little or no assurance that the fractions represent other than complex mixtures.

As a rule, detailed amine acid analyses have been obtained only for the chief globulin fraction, and it has been the custom, for want of more complete information, to attempt to assess the nutritive effect of the total protein of a seed from what is known of this component. The fallacy of this treatment of the data may be illustrated by the case to which attention has recently been called by Jones (36). Arachin, the main globulin of the peanut, has been found to be deficient from the nutritive point of view in both tryptophane and methionine but conarachin, a globulin present in smaller proportion in this seed (8 per cent of the fat-free meal as against 25 per cent of arachin) (37), yields relatively high proportions of these two amino acids (38, 39). Nutritive supplementation therefore occurs when the whole protein of this seed is fed, whereas a deficiency would be apparent if the experiment were restricted to the main globulin. In many, if not most other cases, knowledge is indeed limited to the amino acid composition of the main globulin and, to make the matter worse, few even of

<sup>3</sup> Personal communication from Dr. T. D. Fontaine of the Southern Regional Research Laboratory, New Orleans. The analysis was made by Dr. R. C. Warner of the Eastern Regional Research Laboratory, Philadelphia.

these proteins have been studied save with respect to the more easily determined amino acids. As a result, the efforts to provide analytical information upon which to base a judgment of the nutritive value of the whole protein of such an important food material as the soy bean have been gravely circumscribed in spite of the urgent necessity for such information that has arisen from the present war conditions. This whole problem has been discussed in detail in a recent symposium (40).

It may not be out of place to draw attention to the nature of the mixture of proteins that an extract of a seed may be expected to contain. Aside from the main storage protein or proteins, usually globulins in the case of the dicotyledonous seeds, there should be present components that represent the proteins of the embryo and also the enzymes of both cotyledon and embryo tissue. Furthermore, there is no reason to assume that the whole of the cotyledon protein is storage protein, there are presumably residues of cytoplasmic and nuclear protein, derived from the cells of the cotyledons, some of which may be brought into solution during the process of extraction, and in addition the proteins derived from the tissue of the seed coat must be considered although these doubtless form a relatively small part since the seed coat seems to consist mostly of substances allied to the carbohydrates (41), and to contain little nitrogen.

In general, it is impossible to bring into solution all of the nitrogen of a seed when solvents appropriate for proteins alone are used. As an example, the experience of Smith and Circle (42) with fat-extracted soy bean meal may be quoted. These observers have studied the proportions of the seed nitrogen that are dissolved or "dispersed" over a wide range of conditions. In the absence of added salt, the curve for the soluble nitrogen passed through a rounded maximum at about 85 per cent in the vicinity of pH 2, dropped rapidly to a rounded minimum at a level of about 10 per cent at pH 4, which is in the isoelectric range of the main protein components, and rose rapidly between pH 5 and 7 where it flattened out at about 85 per cent, this quantity increasing gradually to 95 per cent at pH 11 to 12. In the presence of added salt, a family of curves was obtained depending on the concentration of the sodium chloride employed. At low salt content (0.01N), the curve differed but little from that with water, with 0.1 N salt the maxima were a little lower and the minima a little higher, with 0.5 N salt the maximum at pH 2.5 was at 70 per cent, the minimum at pH 4.2 was at 42 per cent, and the curve then rose rapidly and followed a nearly straight line course from 73 per cent at pH 6 to 92 per cent at pH 11. The curves as a whole provide the most complete picture available of the basic solubility relations of the proteins of this seed and somewhat similar data have recently been published for the proteins of the peanut by Fontaine and his associates (43). It should be emphasized perhaps that the determinations were made with the use of entirely empirical and conventional method of extraction, but the observations are nevertheless strictly comparable *inter se* and doubtless represent a fairly general phenomenon.

Of the 10 to 12 per cent of the soy bean nitrogen that passed into solution in water at pH 4, about half was not rendered insoluble by reagents that are

ordinarily used to precipitate protein. This quantity therefore furnishes an approximate measure of the non-protein soluble nitrogen of the seed, such substances as asparagine, choline, and adenine together with amino acids and simple peptides being presumably represented. Furthermore, that part of the nitrogen in this fraction that was precipitated by the reagents tried may be regarded as a rough measure of the proportion of the true water-soluble proteins of the seed, that is, of the proteins that conform to the usual definition of the term albumin. The measure is indeed rough since the solution analyzed contained that part of the isoelectric protein that was soluble in the volume of solvent and at the temperature and salt concentration employed, while no allowance is made for the albumin-like proteins that may have been present in the precipitate either adsorbed upon insoluble protein or in combination with it.

The part of the seed protein that resisted extraction with saline solvents at alkaline reactions presents a difficult problem. In the first place, the quantity observed would be expected to be a function of the success of the grinding operations to which the meal had been subjected. Only if there were a presumption that nearly all of the cells had been ruptured and that time had been allowed for adequate diffusion of the contents could one assume that this fraction represents definite insoluble components. However, Smith, Circle and Brother (44) have furnished data that indicate that their grinding operations were effective, and there seems little reason to doubt that much of the insoluble residue represents protein different in nature from the soluble material. The quantity is perhaps too great to make reasonable the assumption that it represents the protein components of the embryo although these might well be in part included since they are presumably allied in behavior to the proteins of leaves, such proteins are usually difficult to extract with the customary protein solvents after the tissue has been dried.

It is superficial to dismiss this insoluble part of the seed protein as representing a portion of the main protein that had become denatured during the operations of grinding and extraction. Such products are usually soluble at slightly alkaline reaction, whereas 5 per cent or more of the total seed nitrogen remained insoluble even when a solvent as alkaline as pH 11 was used.

The main protein components of the soy bean were obtained from the extract by Smith and his co-workers by isoelectric precipitation at about pH 4.1. They describe conditions under which they recovered approximately 84 per cent of the seed nitrogen in the form of a dry product suitable for many industrial applications, and presumably also satisfactory for the investigation of the nutritive properties of this part at least of the total protein. Nevertheless, that material of this kind is probably far from homogeneous from the point of view of the protein chemist was made apparent by some recent unpublished experiments carried out in this laboratory. The object of these was to secure by modern methods, from a known variety of soy beans, specimens of protein that should represent as nearly as might be the globulin glycinin described many years ago by Osborne and Campbell (45). Accordingly soy beans of the variety

Illini<sup>4</sup> were ground and extracted with petroleum ether, whereby most of the fat content was removed, and, after the solvent had evaporated, were further ground until all passed through a fine sieve. The meal contained 7.00 per cent of protein nitrogen and 0.37 per cent of non protein nitrogen. About 91 per cent of the protein nitrogen could be brought into solution when the material was thoroughly extracted in a Waring Blendor with 6 per cent sodium chloride solution adjusted to pH 7.1 to 7.5 by the addition of barium hydroxide. The suspension was subsequently centrifuged in a cup centrifuge and the residue washed. The solution, or "dispersion," so obtained was extremely turbid and remained so even after passage through a Sharples supercentrifuge. This last procedure threw down, in the form of a sludge in the centrifuge bowl, a product high in ash as well as nitrogen, and the somewhat clarified solution contained only about 75 per cent of the seed protein nitrogen. Owing to the impossibility of obtaining clear solutions either by centrifugation or filtration, after extraction of the meal by this technique, experiments were next carried out in which the meal was repeatedly ground in suspension in either 6 or in 10 per cent sodium chloride solution adjusted to pH 7.1 to 7.5. The suspension was thickened by the addition of sufficient filter paper clippings to make a soft coherent mass, was again passed through the mill and was then formed into cakes wrapped in drilling and pressed between steel plates in the hydraulic press. The solvent of lower salt concentration still gave a turbid solution after the extract had been passed through the supercentrifuge, but that of 10 per cent concentration yielded a solution that was markedly superior in appearance, being almost clear. This method of extraction is one that has repeatedly been found to be effective with many kinds of seeds but, in the present case, the clarified extract again contained only about 75 per cent of the protein nitrogen. There was little sludge deposited in the Sharples centrifuge bowl and it was obvious that the apparently more effective treatment with the Waring Blendor, with respect to the proportion of the protein nitrogen that was extracted, merely represented the dispersion of a protein component, or more accurately fraction, that was so far from being in true solution as to be easily sedimented in the supercentrifuge. Its physical properties were different from those of the main globulin which was present in true solution, but there seems little doubt that a similar material would be present in preparations of the type discussed by Smith and his associates.

Inasmuch as complete clarification of the solution is an essential prerequisite to the preparation of a protein fraction free from non protein contaminants, attention was next turned to the use of even more concentrated saline solutions for the extraction. It was found that saturated sodium chloride solution, adjusted in reaction after being mixed with the meal as before, gave extracts that were perfectly clear both by transmitted and reflected light after being passed

<sup>4</sup> The beans were obtained through the courtesy of Prof. W. C. Rose of the University of Illinois; they were designated "Illini beans certified M18." The experiments were carried out by Dr. G. W. Pucher and Mr. L. S. Nolan of this laboratory.



slowly through the supercentrifuge. They deposited only a trace of sludge in the bowl. Obviously this solvent failed to bring into suspension or solution those components of the meal to which the unusual turbidity of ordinary extracts is due. On the other hand, it extracted only about 60 per cent of the total protein of the seed, a result not unlike that of Smith and Circle who found that saturated sodium chloride solution extracted only 73.6 per cent of the total nitrogen of Illinois soy beans under the conditions of their experiments. Nevertheless, extracts prepared in this way proved to be admirable for the isolation of the main globulin fraction. After dialysis, and adjustment of the reaction of the supernatant solution to pH 5.0 by the addition of sufficient acetate buffer of pH 4.7 and ionic strength 1.0,<sup>5</sup> the protein separated in a form that could be readily centrifuged in the cup centrifuge, and could be purified by being suspended in a liberal volume of 10 per cent sodium chloride solution and redissolved at pH 8.0 by the addition of 0.2 N sodium hydroxide. This solution was then clarified and again dialyzed, and the protein was flocculated at pH 5.0. After being dehydrated with acetone and dried with exposure to the air, the preparations contained between 16.9 and 17.0 per cent of nitrogen and 0.2 per cent of ash, calculated moisture free. Such preparations represent as nearly as can be ascertained the "glycinin" described by Osborne and Campbell, but the product derived from Illinois soy beans differed in nitrogen content from the one described by those workers. Their glycinin contained 17.53 per cent of nitrogen corrected for ash and moisture although its solubility relationships were closely similar. Evidence given by Csonka and Jones (46) suggests that the main globulin fractions obtained from soy beans of different varieties differ from each other in nitrogen content, a point which is in itself indicative of lack of homogeneity, and this may be the explanation of the failure of the present experiments to duplicate Osborne's preparation. The material was, however, found to be identical in histidine content with a preparation made many years ago by the salt precipitation method under Osborne's personal direction (47).

The yield of globulin was in most cases close to 20 per cent of the weight of the fat-extracted meal. Since this meal contained 7.0 per cent of protein nitrogen, it may be assumed that about 40 per cent of protein was present in it, thus the preparations of purified main globulin obtained represent only about half of the total protein of the seed. Although low, this yield is higher than that recorded by Osborne and Campbell who obtained only 16 per cent of the meal as globulin that could be separated by dialysis.

Obviously the total protein of soy beans represents a complex mixture of components. It was found possible to secure preparations in a yield as high as 30 per cent of the meal by treatment of a clarified and dialyzed extract with diluted alcohol to a final concentration of 40 per cent at pH 5.0, but the product was low in nitrogen and high in ash content. The product undoubtedly represented a mixture of many components since there were present not only the main globulins but also the more soluble globulins that separate incompletely when

<sup>5</sup> The buffer solution is made by mixing 250 ml. of 4.0 M sodium acetate with 75 ml. of glacial acetic acid and diluting to 1000 ml.

the dilute dialyzed solution is adjusted to the isoelectric range as well as the proteins originally soluble in water alone. The preparations contained much denatured and insoluble material and their behavior shed little light upon the general nature of the proteins of this seed. Nevertheless, suitable modification of this technique may lead to effective fractionation.

Legume seed proteins are far less satisfactory to deal with in the laboratory than are the proteins of the oil seeds, and the experiments just described furnish an illustration of this fact. Yet it was from a legume seed, the jack bean, that Sumner was able to isolate crystalline urease (48), and his achievement in obtaining at least three other protein components in crystalline form (49) remains as a demonstration that the isolation of what are presumably the individual proteins of legume seeds is not a hopeless undertaking, difficult though it may be.

This section may be concluded with some remarks on the directions that it would seem desirable for future research on seed proteins to take. Two quite different motives make such research essential. In the first place, those who are interested in nutrition, especially in the vitally important problem of the selection of sources of plant proteins to serve as substitutes for the more highly esteemed and widely used proteins of animal origin, require comprehensive and trustworthy data on the amino acid composition of the whole protein of many kinds of plant seeds. Much of the available data refer, as has already been mentioned, only to specimens of the more easily prepared proteins and these may represent as little as one-half of the total protein that is made available on ingestion of the seed meal or of protein rich concentrates from it. Techniques must therefore be developed whereby samples can be obtained that represent the whole protein, or at least a large part of it, in a form so purified from carbohydrate and other contaminants that the fractional losses of amino acids that occur during hydrolysis will not significantly affect the analytical results. Whether one or more than one fraction is secured is immaterial so long as the quantitative relation to the seed meal is ascertained and the fundamental data established that will permit the calculation of the amino acid composition of the entire seed. The opinion is widely held that the hydrolysis of seed meals themselves, or of grossly impure protein concentrates from them, is a feasible approach to the solution of this problem. Control analyses are occasionally presented to show that hydrolysis of a protein in the presence of starch or of a sugar results in small if not wholly negligible losses of certain amino acids (50). Unfortunately such controls are not entirely convincing. They assume that no other kind of component is present in the material hydrolyzed which can interfere by giving rise to humin at the expense of the amino acids. Yet Chubb (28, p. 140) and Lugg (51) have both strongly emphasized the danger that arises from the presence of contaminants of plant origin in samples of proteins subjected to hydrolysis. The special contaminant with which they were concerned is almost invariably found in preparations of proteins from the leaf, and they carefully point out that its chemical nature is still unknown. However, the evidence suggests that it may be allied with the pentosans or pectins or possibly with the substances classified as mucilages. At all events experi-

ments carried out in this laboratory (52) have shown that the humin nitrogen produced during the hydrolysis of edestin may be increased from a mere trace to as much as 9.7 per cent of the total nitrogen if furfural is added, or to 5 per cent in the presence of pectin, this last quantity being increased still further if cellulose were also added. The loss of 5 per cent or more of the protein nitrogen in the humin cannot but have serious effects upon the apparent amino acid composition, especially if it is remembered that the losses probably fall unevenly upon the various amino acids present.

The other motive for further research upon seed proteins is admittedly more academic, but it would seem that the time is now rapidly approaching when the whole problem of the nature of the seed proteins should be reinvestigated with the aid of the modern physico-chemical techniques that have made possible the striking advances of recent years in such a field as that of the blood proteins. The system of protein components in an extract prepared from a seed meal is quite different in properties, especially in solubility, from such a system as that in blood, or milk, or an extract from a gland, but there is no reason to assume that its details cannot be worked out equally as successfully, although doubtless the methods will have to be modified to meet the special needs of the case. The inapplicability to the seed globulins, for example, of the present procedures for the measurement of mobility in the electrophoresis apparatus is indeed a challenge to those concerned with the development of this extremely useful device. The application of modern theories of protein solubility to the problem of the separation of these proteins from each other would appear to offer no insuperable fundamental difficulties.

Research in the past, both with animal and with plant proteins, has been too often restricted to the investigation of a single or at most a small group of components of a given tissue. Where this component has special physiological activities or interests, this is justifiable, and in those cases where the component has favorable physical and chemical properties, gratifying success has often attended the efforts. But the time has come when a broad and far more detailed view of the chemical composition of living organisms is becoming essential to our understanding of their behavior, and it is well to remember that the simple plant seed, the ultimate source of all foodstuffs, is one of the most important organisms with which mankind is concerned.

**LEAF PROTEINS** Knowledge of the normal protein components of plant tissues other than the seeds is in a far less satisfactory state than is that of the seed proteins. It is only recently that a moderate degree of understanding has been obtained of the relationship between the chlorophyll and the chloroplastic proteins in leaves, and that trustworthy information on the composition of the proteins of the leaf cytoplasm has become available, but there is little that can be considered even yet as systematic information about these substances. With respect to the proteins of other tissues there is direct evidence from chemical studies that proteins are to be found for example in bark (53), or in fruits (54), or in rubber latex (55), and some of these products have been subjected to more or less conventional investigation for amino acids although little has been ascertained about their solubility relationships in the native state.

It is an interesting commentary upon the effectiveness of modern physico-chemical methods of research on proteins that the only leaf proteins concerning which a truly comprehensive body of knowledge should have been accumulated in recent years are the virus proteins, substances that are formed in leaves as a result of specific diseases and which are themselves the agents whereby these diseases are transmitted. In this field alone has the full armamentarium of modern physico-chemical research been employed by skillful and enthusiastic workers and the results have indeed been striking. The reasons for this are not far to seek, the virus proteins of diseased leaves were promptly shown to be type substances characteristic of virus disease in general and their investigation was accordingly greatly stimulated by the medical implications of this fact. Their enormous molecular weight and convenient solubility made it possible to isolate them by physical methods, and their physiological activity even in most extreme dilutions rendered tests for their presence a fairly simple matter so that their behavior could be far more precisely followed than is the case for almost any other kind of protein. Thus we are today in the anomalous and logically indefensible position of knowing a great deal more about the proteins of leaves that are suffering from a serious disease than we do about those of normal and healthy leaves. Knowledge of animal disease, especially that of man, rests securely upon a foundation of knowledge of normal anatomy, physiology, and biochemistry although botanists have provided adequate anatomical information with respect to normal plants, far too little has been learned of their physiology and biochemistry.

Examination of a leaf under the microscope shows it to consist of cellular tissue with clearly defined walls. The cells are filled with a more or less jelly like colorless material called cytoplasm, which contains what are termed inclusions of several different kinds. Most important from the biological point of view is the nucleus, the site of the chromosomal material, but the most conspicuous are the chloroplasts. These are tiny green structures present in enormous numbers and in them the pigments of the leaf are concentrated. In addition, particularly in older cells, a central region filled with a clear liquid of low viscosity, and termed the vacuole, is to be distinguished. These different anatomical structures imply the presence of different chemical structures and chemical tests indicate that proteins are present in each of them. One might expect, therefore, that chemical examination of leaves would reveal several kinds of proteins of which two would be found in large proportions. These would represent the protein associated with the green pigment the chloroplastic protein, and the other would be free from pigment and would represent that of the cytoplasm. In addition, if techniques were devised to separate the very small proportion of the nuclear material, one would anticipate that it would be allied in behavior and composition with the nuclear material from the better known animal sources, and would be found to be rich in nucleic acid. Lastly, if the vacuole fluid could be separated, one might expect to find that it contains a little protein in solution although from the low viscosity this could not account for any large part of the total leaf protein.

*Chloroplastic protein* That the chlorophyll of the green leaf is present in

some sort of combination with substances of high molecular weight has long been suspected Willstätter (56) in 1922 pointed out that the solubility behavior of the pigment in the leaf suggests that it is not free in molecular solution but is in large part in what he held to be an adsorbed condition Lubimenko (57) at about the same time was willing to go further and postulated a probable chemical combination between the pigment and the protein component of the chloroplast Noack (58) in 1927 provided strongly suggestive evidence of this He developed a technique for grinding leaves with sand, centrifuging off the debris, and then subjecting the turbid green fluid to high-speed centrifugation whereby a sediment was obtained that appeared to consist chiefly of the chloroplasts themselves although in a shrunken condition He noted that the characteristic red fluorescence of the chlorophyll in this sediment was extinguished when a sample was heated for a few seconds to a temperature that exceeded 70° although it returned if the heating were prolonged Both protein and the fluorescing chlorophyll were precipitated by the addition of ammonium sulfate or of lead acetate to a suspension of the sediment, but the fluorescence was again extinguished if the precipitate were heated, and treatment of the sediment with proteolytic enzymes likewise led to extinction He interpreted these experiments to mean that the chlorophyll is normally combined with or adsorbed upon the protein in such a manner that denaturation or digestion liberates it whereupon it passes, in the aqueous medium, into colloidal aggregates that no longer show detectable fluorescence The return of the fluorescence after more prolonged heating he considered to be due to solution of the chlorophyll in waxy components of the tissue, a view later supported by the experiments of Smith (59)

These observations were followed up ten years later by Menke (60) in Germany and by Granick (61) in this country Menke employed a differential centrifugation technique with 0.66 M primary potassium phosphate as fluid phase, and, by discarding the top and the bottom layers of the sediments, secured material 99 per cent of which he thought to consist of the chloroplasts derived from the spinach leaves used Analyses showed that the solids of this product contained 47.7 per cent protein ( $N \times 6.25$ ), 37.4 per cent lipid, 7.8 per cent ash, and 7.1 per cent undetermined material The lipid fraction, obtained by extraction with ether followed by ether-alcohol, was extremely complex in composition In addition to chlorophyll-a and -b, carotin and xanthophyll, fatty acids, glycerides, phosphatides, sterols, and wax-like components were said to be present<sup>6</sup>

Preparations that represented concentrates of the protein components of the chloroplasts, and termed "chloroplast substance" by Menke, were obtained, among other ways, by treatment of the turbid suspension with one-quarter of its volume of saturated ammonium sulfate solution The flocculent green precipitate that separated was found to contain 56.4 per cent of protein ( $N \times$

<sup>6</sup> Chibnall and his associates have carried out careful analytical studies upon leaf lipids Their data, which agree with these statements of Menke completely, are summarized in Appendix III of his published Silliman lectures (28, p. 284)

6.25), 31.9 per cent of lipid, 4.7 per cent of ash, and 7.1 per cent of undetermined material. After being freed from lipids, the residue consisted chiefly of presumably denatured protein of a corrected nitrogen content of 14.6 per cent which was insoluble in water, dilute acid, alkali, or salt solutions, but could be dissolved in part in 50 to 60 per cent alcohol in the presence of a little alkali. Extraction with very dilute aqueous alkali removed a small proportion of a phosphorus-containing protein component which apparently contained nucleic acid. The residual material was still, however, contaminated with carbohydrate, and its behavior was reminiscent of that of certain preparations obtained in 1920 from alfalfa leaves by Osborne and Wakeman (25) and in 1926 by Chubb and Grover (62). Subsequently Menke (63, 64) provided analytical information upon the content of chlorophyll as well as upon the inorganic constituents of these preparations.

Granick (61, 65) introduced what seemed to be an improvement in the method of preparing chloroplasts by grinding the leaves in the presence of hypertonic sugar solutions. With the use of 0.5 M glucose or sucrose, he was able to secure sediments of chloroplasts in which the proteins were apparently not denatured and which remained normal in appearance for several hours after isolation. Tests showed them to be able to produce oxygen in the presence of light for several minutes. Determination of the quantity of chlorophyll *a* + *b* in the isolated sediment and in the whole leaf permitted an estimate of the relative proportion of the total chloroplasts of the tissue that had been isolated, and from this ratio and the nitrogen content of the sediment it was possible to obtain a reasonable measure of the relative proportion of chloroplast nitrogen in the leaves. For the tobacco and tomato leaves that he studied, he thus found that from 30 to 40 per cent of the *total nitrogen* of the leaf is present in the chloroplasts. Of the nitrogen of the chloroplasts, 13.2 per cent was present in the ether alcohol extract of the lipids, 6.7 per cent was soluble in trichloroacetic acid solution, and the remaining 80.1 per cent therefore presumably represented protein. The lipid nitrogen consisted of chlorophyll nitrogen to the extent of about 10 per cent of the total chloroplast nitrogen and the balance of 3.2 per cent was assumed to represent such components as phosphatides. It was further calculated that from 35 to 45 per cent of the *protein nitrogen* of the leaf was present as the protein of the chloroplasts, thus leaving from 55 to 65 per cent for the other proteins, chiefly those of the cytoplasm. These estimates, while admittedly approximations, furnish the orders of magnitude of the quantities concerned and have been substantially confirmed by Neish (66) who worked with several other species and extended the observations to a consideration of the inorganic components. This worker, however, employed water extracts, or rather suspensions, and noted that the chloroplasts themselves swell and disintegrate in water with granulation of their contents. The preparations he studied consisted of this granular material which was flocculated by the addition to the suspension of calcium or magnesium salts in a concentration of 0.1 M. They therefore more closely resembled the "chloroplast substance" of Menke than the presumably intact chloroplasts studied by Granick.

Hanson (67) in Australia has also confirmed the observations of Granick, in particular the point that the chloroplast proteins make up about 36 per cent of the total protein of the leaves of a grass species. In addition he concerned himself with the weight ratio between the protein and the chlorophyll in the chloroplast. He found this to vary from 4.5 to 5.4 under various metabolic conditions.

Smith (59) has applied refined spectroscopic techniques to the study of the properties of extracts prepared from spinach and *aspidistra* leaves by grinding the tissue with slightly alkaline buffer solutions. Thus, although he occasionally speaks of the results as applying to the chloroplasts, his preparations were doubtless suspensions of the granules derived from the disintegrated chloroplasts and were more nearly like those of Menke and of Neish. He confirmed early observations such as those of Hagenbach (68) that the absorption band in the red shown by chlorophyll as present in the leaf is shifted slightly towards the blue if the pigment is extracted from the leaf with organic solvents. He also noted a similar shift when his extracts were treated with reagents, or by procedures, that bring about denaturation of the protein. The observations with detergents were especially striking. If digitonin were added to the turbid leaf extract, this at once became clear and showed the characteristic shifts in the spectrum. The behavior of the protein in the solution was likewise greatly modified. It could no longer be precipitated even by saturation of the solution with ammonium sulfate, but after prolonged dialysis, whereby most of the detergent was removed, could be precipitated by the addition of one-tenth of the ammonium sulfate necessary for saturation of the solution. This last property distinguishes such material sharply from that of the protein in the original untreated extract. Careful chemical analyses of products purified by repeated salt precipitation or by differential centrifugation showed that the protein nitrogen content was 7.4 per cent, the protein content thus being  $(N \times 6.25)$  46.5 per cent, remarkably close to that of similar preparations of Menke. The chlorophyll content was 7.86 per cent and thus 100 parts of protein were associated with approximately 16 parts of chlorophyll. This figure differs slightly from the ratio found by Hanson, but this worker employed chloroplasts, isolated by the Granick procedure, which might well contain a surplus of protein other than that combined with pigment.

Smith has also studied the effect of other detergents than digitonin, especially that of sodium dodecyl sulfate (69) which was found to convert the chlorophyll in the protein complex into phaeophytin by removal of the magnesium at a rate dependent on the hydrogen ion concentration. At constant pH, the rate was proportional to the concentration of the detergent. Evidence that the protein component is denatured by the treatment was obtained from its solubility behavior. The combination between pigment and protein was not broken, however, in spite of this change, and it was accordingly concluded that magnesium plays no part in the linkage between protein and green pigment.

Considerable light was shed upon the relationship between the protein and the pigment in the presence of detergents by the study of Smith and Pickels

(70) of the behavior of the solutions in the ultracentrifuge. Untreated extracts of the chloroplast material were found to sediment at relatively low speeds (2500 to 3000 r.p.m.) with a purely random spread of particle size. Thus the original extracts are in fact suspensions of particles that are not in true solution. The addition of detergents to these suspensions converted them into brilliantly clear solutions, but the effects of the several reagents were different. The apparatus permitted the observation of sedimenting boundaries both by light absorption, thus showing the behavior of the pigment or pigment-protein complex itself, and by a refractive index gradient system that revealed the behavior of the dissolved protein in the gravitational field. Furthermore, the light absorption measurements could be made both with red and with violet light filters thus permitting discrimination between the behavior of the carotinoid pigments and the chlorophylls.

When digitonin, sodium desoxycholate, or bile salts were added to the extracts, evidence was found for the decomposition of the protein-chlorophyll complex. The situation with the first of these substances was complicated by the fact that digitonin itself is present in micellar form (71). There was a light absorption boundary which indicated that these micelles combined with or adsorbed the chlorophyll. However, a second boundary was observed from the refractive index gradient picture which was not present in the absorption picture, and this sedimented at a rate corresponding to a sedimentation constant of 13.5 S. With sodium desoxycholate and bile salts, there was no evidence whatever by light absorption for the sedimentation of the pigments, although the refractive index system showed the presence of a boundary which sedimented at the same rate as that just mentioned. Accordingly it was clear that all three detergents dissociate the pigment from the protein, which then sediments separately, the constant being the same in all three cases.<sup>7</sup>

The effects of sodium dodecylsulfate were widely different from these. The removal of magnesium from the chlorophyll has already been mentioned, a reaction that is rapid in the presence of acid being complete in a few minutes at pH 4.8 although requiring several hours at pH 7.5, and a day at pH 8.9. At any of these reactions, however, the pigment sedimented together with the protein, the boundary observed by light absorption being identical with that observed by refractive index increment. Furthermore, the light absorption boundaries seen in the two regions of the spectrum were likewise identical indicating that both the chlorophyll and the carotinoids were attached to the protein, either to particles of the same molecular size and shape or to the same particles.

The rate of sedimentation was much slower than that of the protein component observed in the presence of the other detergents. The sedimentation constant was 2.56 S when the protein concentration was low and the detergent concentration was 0.25 per cent. At higher protein concentration (about 1.3 per cent) and a ten times higher concentration of detergent, the sedimentation

<sup>7</sup> An incidental observation was made that in concentrated urea solution the pigment is likewise dissociated from the protein.



constant was 1.69 S indicating still further dissociation of the protein molecules although the pigment remained attached to the protein

In the absence of measurements of the diffusion constant, calculations of the molecular weight are impossible, nevertheless Smith and Pickels offered the estimate that the protein of sedimentation constant 13.5 S found in the extracts that had been treated with digitonin or bile salts would have as a minimum a molecular weight of 265,000. The particles present when sodium dodecylsulfate is added are smaller although apparently reasonably homogeneous in size distribution, those observed in a 2.5 per cent solution of detergent being about half the weight of those in 0.25 per cent detergent.

To summarize the chemical information that has been obtained from these recent investigations of chloroplasts and their contents, it should first be mentioned that the chloroplasts are present in the cells of leaves and other green tissues in relatively enormous numbers. Estimates have been made that run to the order of several hundred thousands per square millimeter of leaf surface. An excellent review of the available information concerning them has been given by Weier (72). From many points of view, the chloroplasts bear an analogy to the red cells of blood. Since they contain the chlorophyll of the leaf, they are the locus of the reactions by means of which carbon dioxide is reduced, starch is formed, and oxygen liberated. Accordingly they are structures provided with all of the enzyme systems necessary for these activities. They are apparently bounded by a semi-permeable membrane and, when freed from the cellular tissue in the presence of water or dilute salt solutions, swell and break open thereby liberating their contents in the form of granules that readily pass into colloidal suspensions. On the other hand, if the leaf cells are ground in the presence of hypertonic solutions of sugars, the chloroplasts can be secured in a not too seriously damaged condition and they preserve some at least of their functions for a short time. It is obvious therefore that chloroplasts are available for chemical study and, although it is unfortunately much more difficult to secure preparations of them than it is of the red cells of blood, there is reason to believe that many of the chemical and physiological methods that have been employed in the study of red cells are equally applicable to chloroplasts.

The observations that have been made indicate that the chemical system within these structures is very complex. Considered in the gross, the chloroplasts contain about 35 per cent of the total protein of the leaves from which they are prepared, all of the chlorophyll and probably the greater part of the other pigments and much of the lipids. They are high in inorganic constituents (64, 66), and Menke's analyses suggest the presence of carbohydrates as well. Certainly starch is present when their photosynthetic functions have been exercised immediately before isolation.

Exactly how complex the protein system of the chloroplast may be does not appear from the evidence at hand. Menke observed a small proportion of what seemed to be a nucleoprotein component and one would anticipate the

presence of a certain proportion of enzyme proteins<sup>8</sup>. The bulk of the protein, however, appears to consist of components that are perhaps to be classified as lipo-proteins, the evidence now seems complete that the chlorophyll is combined with this protein as a so-called prosthetic group and the same may be true of the carotinoids. It is possible that both types of pigment are combined with the same protein molecule.

Attempts have been made (63, 67) to deduce stoichiometric ratios between the protein and the chlorophyll but these have been predicated on wholly unsubstantiated assumptions. Such ratios will have significance to the chemist only when preparations of the lipo-protein have been made that meet the criteria for a pure homogeneous substance. The molecular size of the chlorophyll protein is still unknown although Smith and Pickels' estimate gives an order of magnitude. At all events, the protein is several times larger than hemoglobin and is very much smaller than the virus proteins of leaves studied by Stanley and his associates.

Future investigation of this type of leaf protein will doubtless be immeasurably assisted when a clearer understanding of the nature of lipo-proteins in general has been obtained. It is to be hoped that techniques now available and in process of development for the study of the lipo-proteins of blood serum may find an application in this field.

*Cytoplasmic protein.* The isolation of the proteins of the cytoplasmic substance of the living leaf cell presents a chemical problem of the greatest difficulty. Here at least one can be relatively certain that he is dealing with a complex mixture for the cytoplasm is the seat of the chemical reactions that are associated with life itself. It is accordingly a dynamic system and there is every reason to suppose that the proteins are among the most important and perhaps the most reactive of the substances which share in the processes that take place. Present day views of the significant part played by the proteins in the chemical reactions of the animal body have been formulated for the most part as a result of the fundamental researches of Schoenheimer and his associates with isotopic nitrogen. Far from being a stable structure which remains for a long time unchanged, the individual cell protein molecule is today recognized as a unit which in all save the most inert tissues has a remarkably short life. That a similar situation probably exists in the plant cell is a logical assumption, and evidence to this effect was obtained in 1940 by Vickery, Pucher, Schoenheimer and Rittenberg (73) who observed that isotopic nitrogen is taken up by the proteins of the tissues of both tobacco and buckwheat plants in excess of the quantities that could reasonably be accounted for by growth alone during the experimental period. Similar results have been reported by Hevesy, Linderstrom Lang, Keston and Olsen (74) in the case of the sunflower plant. Furthermore the unique reactivity of glutamic and aspartic acids in the nitrogen exchange reactions of animal tissues was also found to characterize their behavior in the

<sup>8</sup> Neish has demonstrated catalase and carbonic anhydrase functions in material prepared from disintegrated chloroplasts.

plant This last point is not, however, a matter for surprise because of the unique position that the amides asparagine and glutamine have long been recognized to hold in the metabolism of plant tissues

Accordingly, although one can doubtless learn to isolate preparations that represent protein components of the cytoplasm, there is no reason whatever to expect such preparations to be homogeneous from the chemical point of view Quite the contrary is to be anticipated Although chemical examination may give information on the over-all average composition, it will reveal little concerning the individual substances present, and because of the instability of these proteins it is improbable that the preparations secured by any of the methods hitherto suggested will resemble the original material of the cell at all closely in its physical and chemical properties

The isolation of preparations that represent a part at least of the protein of leaf cell cytoplasm in a form uncontaminated by proteins derived from the chloroplasts was probably first accomplished by Chibnall in 1923 (75) It had long been known that the vapors of chloroform or ether, or aqueous solutions of these and similar substances, would so affect the permeability of plant cell membranes as to permit the vacuolar fluid contents to exude The reaction is irreversible, if allowed to proceed for more than a few minutes, and results in the death of the cell In his previous work, Chibnall (27) had made frequent use of ether water as a plasmolyzing reagent in order to facilitate extraction of proteins from leaf cells, but in 1923 he noted that when leaves were immersed directly in ether, they at once lost their turgidity, and that mere pressure with the fingers would suffice to express a large part of the fluid contents This observation revealed the possibility of treating whole leaves in such a way as to remove the vacuole fluid without rupture of the cell walls

Chibnall found that, after treatment of the leaves in bulk with ether, it was possible to squeeze out most of the vacuole fluid with the hydraulic press, and to wash away the remainder by alternately allowing the tissue to imbibe water and pressing it out several times The object of this operation, from the chemical point of view, was to remove water-soluble components before attempting to bring the proteins into solution The vacuole fluid so obtained contains very little protein in solution but may contain 25 per cent of the total leaf nitrogen Furthermore a substantial proportion of the inorganic constituents which might later contribute to the ash content of the protein preparation could thus be removed After this treatment, the residual tissue mass was left with its cells for the most part unbroken and still containing the jelly-like cytoplasm with its inclusions of chloroplasts and other formed elements Moreover, it was in a physical condition that made the grinding operation for the rupturing of the cell walls relatively easy After being first ground in a meat grinder and then repeatedly passed in dilute suspension in water through a plate-type mill to afford maximum shearing action on the cell walls, the tissue residue was removed with the aid of a silk screen and a green turbid suspension that contained much of the protein of the leaves was secured This was filtered through a thick pad of paper pulp whereby the green pigment and all of the turbidity were re-

moved. It was at this point that Chibnall accomplished the fundamental separation of the two main types of protein components of the leaf. The chloroplastic material including both chlorophyll, other lipids, and protein, being present under these conditions as a suspension of particles, as has already been pointed out in connection with the recent work of Smith and Pickels, was removed by the efficient pulp filter while the filtrate contained that part of the cytoplasmic protein that had passed into true solution. A qualification is perhaps necessary here, the filtrates obtained were clear, with only a small Tyndall effect and gave every appearance of being true solutions but, until such extracts have been studied by modern physical methods, it cannot be asserted that they are, in fact, true solutions of cytoplasmic protein. Assuming this for the present, however, the next step was the flocculation of the protein by the addition of the requisite amount of dilute acid, in general, such proteins are least soluble in the range pH 4 to 5. The precipitated protein was removed and was usually further purified by being dissolved in the least possible amount of dilute alkali and reprecipitated with acid. It was finally collected and washed and dehydrated with graded strengths of alcohol. In successful preparations, the first alcohol washes should contain at most traces of green color, if more were present the filtration step for the removal of the chloroplastic material must have been inefficient. Final extraction with ether as a rule sufficed to remove a residual trace (1 to 2 per cent) of lipid.

Chibnall has applied this method to the examination of a wide variety of leaf proteins, especial attention being given in more recent years to the grasses because of their agricultural importance. Some of the preparations were practically free from non protein contaminants as evidenced by their high nitrogen content. A preparation from spinach leaves (76) contained 16.25 per cent of nitrogen and one from *Vicia faba* leaves (62) 16.77 per cent. Most of them, however, were in the range from 14 to 16 per cent and doubtless contained non protein material although the nature of this and whether it should be regarded as a contaminant or as an intrinsic part of this kind of protein is still to be determined (see 28, p. 138 and p. 278 for a discussion of this point).

A vitally important feature of these experiments, and one that has scarcely been commented upon save by Chibnall himself, is the question of yields. Even in the especially favorable case of the spinach leaves, only about 14 per cent of the leaf nitrogen (19.4 per cent of the leaf protein) was isolated in the form of the cytoplasmic protein and from *Phaseolus multiflorus* leaves only 16 per cent was obtained. In most other cases where successful preparations were obtained, the yields ranged from 1 to 8 per cent, while a number of species were encountered with which the method failed entirely to yield cytoplasmic protein. In the case of the grass proteins to which much attention was given (77), a return was ultimately made to the use of ether water rather than of ether itself as the plasmolyzing agent and many successful experiments were then carried out. In these circumstances too, however, the yields were often disappointingly small and were always erratic, it became clear that some highly important factor was being overlooked or at any rate was not being controlled.

Before going on to discuss the later experiments which threw some light on the situation, it may be well to stop and consider the chemical conditions in the leaves that are being subjected to this process. In the first place, the effect of the ether or ether water upon the semi-permeability of the cell is a phenomenon of both biological and chemical import. When cells treated in this way are examined under the microscope, the entire protoplast is seen to have shrunk away from the cell wall and to be located at one end of the cell. The fluid of the vacuole has mostly passed through it, and the cell is filled with what is presumably a mixture of vacuole fluid and absorbed ether water, if this reagent has been used. The delicate equilibria upon which the life of the cell depends are obviously completely upset, and the phenomena that occur subsequently are clearly in the province of the chemist rather than in that of the biologist. By the successive pressing and washing operations that are next carried out, the vacuole fluid is washed away and the tissue is then thoroughly ground in the presence of a considerable volume of water. During this operation, the proteins of both chloroplasts and cytoplasm pass into what Chibnall speaks of as colloidal solution. What happens to the chloroplasts would seem to be reasonably clear from the results of Menke and others that have already been discussed. It can be assumed that they are disintegrated and that their contents are liberated in the form of the granules that Menke terms chloroplast substance and which Smith and Pickels found were in fact gross particles that had a wide distribution with respect to size. At all events, their practically complete removal by the subsequent efficient filtration operation would be expected. The crux of the question is what happens to the jelly-like mass of the cytoplasm exclusive of the chloroplasts. If this is indeed a thixotropic gel, it is possible that it may be dispersed by the mechanical effect of the grinding and, owing to the dilution with water, may then be converted into a form that will pass through the filter, more or less of it may pass into true solution. Obviously, until such solutions have been examined in the ultracentrifuge and in the electrophoresis apparatus we are scarcely in a position to do more than guess. However, certain points that may have their influence on the outcome of any individual preparation are more or less obvious. In the first place, a part of the gel at this stage may consist of protein that has become denatured as a result of mechanical action during grinding or of the action of surface forces. Chibnall places some emphasis on this possibility and, until we know much more of the physical and chemical properties of proteins of this kind, we shall be unable to assess its validity. But possibly more important is the fact that the proteins of the gel are now exposed to the action of a solvent of very low ionic strength at a reaction that is the result of chance. No buffer save the protein itself is present, and experience shows that these solutions are usually only a short distance removed from the isoelectric range of the proteins since only small quantities of acid are required to flocculate them. It would seem therefore that the conditions that have been reached are those more apt to promote instability of the protein systems involved than stability, and perhaps it is not surprising that success with this method of preparation is sometimes erratic. Experiments to test this view of the situation are clearly required.

However this may be, Chibnall and his associates (78) in 1933 described a technique by means of which some, at least, of the difficulties of the earlier method have been overcome. As a result of a chance observation, they found that ether water that had been used once for the plasmolysis of a batch of leaves was thereafter far more efficient for use in the treatment of subsequent batches. It was noted that the effect of such ether water upon the protoplast was somewhat slower and that it did far less violence to the cells inasmuch as marked shrinkage of the protoplast did not take place. Nevertheless, the permeability of the cell was destroyed as effectively as before and the removal of the vacuole fluid was equally easy. After the tissue was ground, the suspension of the proteins was worked up as usual, but the yield of protein after flocculation of the filtered solution was greatly increased. In a series of some seventeen species, mostly grasses, the yields ranged from 7 to 31 per cent of the leaf protein, with an average of about 18 per cent, and the experiments on repetition were far more satisfactorily consistent than with the earlier method.

Chibnall later pointed out (28) that the chemical conditions, if "used" ether water is employed, are indeed widely different and that the effect of the whole preparation method is changed. Whereas, in the earlier method the clear filtered solution of the cytoplasmic protein showed only a moderate Tyndall effect, the solutions now obtained exhibited this property to a marked degree. Furthermore, appreciable quantities of lipids were present in the preparations and, most important of all, far less protein remained on the filter in combination with the lipids, most of it passed into solution and was found in the filtrate. In other words, the specimens of leaf protein secured by this revision of the technique consisted not only of the soluble cytoplasmic protein but also contained a substantial part of the chloroplastic protein that had been combined in the tissue with the lipids. It appeared possible that the effect of the new reagent was in some way to dissociate the bond between lipid and protein and permit chloroplastic protein to pass into solution.

Chibnall has discussed this still unexplained effect in terms of the wide differences that are to be observed under the microscope between leaf cells that have been treated on the one hand with ether water and on the other with "used" ether water (28, p. 152 ff.). He points out that the new reagent is taken up by the leaves during the process in far larger amounts than is freshly prepared ether water, and offers as a speculation that the reagent, in contact with the outer surface of the protoplast, may in some way peptize the lipid which is acting as a protective colloid to the protein gel. The gel is thereby rendered available to solvents after the cells have been opened by the grinding operation. The effect was shown in the 1933 paper to be due not to a lowered concentration of the ether but to substances which diffused from the leaf cells, that is to say, to admixture with a small proportion of the vacuole fluid.

The new technique made possible the isolation of protein preparations that represented a substantially larger part of the total protein of the leaf cells, and for many purposes, such as the assay of the amino acid composition of these proteins, were a great improvement over the earlier preparations. Analysis of such samples of the whole protein and of the cytoplasmic protein prepared by

the earlier method permitted approximate calculation of the composition of the chloroplastic protein by difference. Nevertheless the observations as they stand offer a challenge to those interested in leaf cell proteins as substances, and it is clear that the new method, inasmuch as it leads to the isolation of even more complex mixtures than before, requires further modification to permit fractionation of the components.

A purely speculative suggestion may be offered for what it is worth in the effort to throw some light on the behavior of the proteins during treatment of the cells by the "used" ether water method. The reagent used for the plasmolysis is in effect a dilute solution of the vacuole fluid to which ether has been added (28, p. 155). Chibnall and his associates (78) describe in detail an experiment in which they made up the volume of the ether water used for a series of batches of leaves by the addition of some of the juice from the press, the ether concentration being maintained by suitable additions from time to time. The significant fact here may be that the subsequent extraction after the tissues had been ground was made not in the presence of a solvent of almost zero ionic strength, as is the case when fresh ether water alone is used, but in the presence of an admittedly small but definite concentration of salt owing to less effective washing away of the fluid contents of the cells. Under these circumstances, one of the chief theoretical objections to the original ether or ether water methods is diminished in force. The extraction is made in the presence of a buffer solution of low ionic strength and the protein systems may be imagined to be thereby rendered somewhat more stable. In addition, such a solution may well be a somewhat more efficient solvent for the cytoplasmic protein.

This speculation leaves out of account the question whether or not that part of the chloroplastic protein that is brought into solution in the filtrate is dissociated from the lipid. The facts appear to be that substantial quantities of lipid do pass through the filter, and Chibnall speaks of the strikingly enhanced Tyndall effect in these filtrates. Moreover, extraction of the flocculated preparations with alcohol and ether is necessary subsequently to remove this lipid. But whether or not it is still in combination with the protein does not seem to be established. Again, the application of modern physico-chemical methods would seem to offer a hope that the situation may be ultimately clarified.

Chibnall's experiments have yielded a great deal of information upon the composition of an array of protein preparations secured from the leaves of many important agricultural species. These data bear directly upon the fundamental question of the nutritive effect of leaf proteins. He pointed out that the amino acid composition of all of these preparations was remarkably uniform from species to species and that the composition was such that good nutritive properties were to be anticipated. Among other points, for example, the long and hotly debated question of the source of the cystine in the fleece of sheep restricted to a diet of herbage was finally settled by the observation that the cystine content of the leaf proteins of such herbage is in fact adequate to account for the cystine in the fleece even of notably heavy wool-producing animals (79).

On the other hand, his experiments as a whole were designed to provide only

the preliminary data required by the protein chemist and the agriculturist. He says "But much remains to be learned, for the existing methods often fail to extract proteins in reasonable degree of purity from leaves of many important groups of plants. Moreover, the leaf proteins, in contrast to the better-known reserve proteins of seeds, are an integral part of a complex and carefully controlled mechanism, the protoplasm, consequently, if the plant physiologist is ever to be in a position to emulate Lawrence J. Henderson's brilliant researches on blood and attempt to explain protoplasmic behavior by considering the protoplast as a physico-chemical system—an ideal to which he has not yet attained—he must know more about the conditions under which the proteins exist in the living cells" (28, p. 120)

**Vacuole protein** Chibnall's ether method provides a technique for the separation of the fluid of the vacuole from the cells without extracting any of the components of the cytoplasm and thus makes possible the chemical investigation of this vitally important part of the system both for proteins and for simpler substances. The technique is immeasurably superior to the crude grinding and alcohol precipitation methods employed to prepare the extracts from alfalfa leaves that were examined for simpler nitrogenous substances by the writer (80) many years ago, and it is extraordinary that so little attention has been paid to the opportunity presented. Most investigators in this field have been content to work with direct alcohol or hot water extracts of the cellular tissues on the assumption that the soluble components are equally well isolated in this way, although no cogent evidence that this is so has been presented.

Chibnall himself, being interested chiefly in the proteins of the leaf, frequently heated the vacuole extract to coagulate such protein as it contained in solution. In this way he established the fact that only a small part of the total leaf protein is ever to be found in the vacuole fluid secured after plasmolysis with ether and with most species none whatever could be demonstrated (28, p. 136). For example, in the spinach leaf (76) only 0.74 per cent of the leaf solids and 1.7 per cent of the leaf nitrogen were found in the heat coagulum and the preparation itself contained only 14 per cent of nitrogen corrected for ash and moisture. A few conventional amino acid analyses of such material are to be found in his earlier papers but, aside from this, there is little in the literature that sheds light upon chemical nature or composition of this part of the protein system.

#### CONCLUSION

The present discussion of the proteins of plants has been presented entirely from the point of view of a protein chemist interested in proteins as chemical substances. Emphasis has therefore been placed upon the problem of their differentiation and purification in the hope that, when sufficient has been learned of them, adequate chemical and physical characterizations can be made. Only when we have learned about the properties of these components of the systems that carry out the functions of the living cell shall we be in a position to learn what these functions really are. Nevertheless, the writer is keenly aware that this treatment of the subject is entirely one-sided and distorted for it is



the *systems* of components that carry out the functions and thus the protein chemist is only one of the team of workers, both chemical and biological, who must combine their best efforts in order that we may ultimately understand what is going on in the living cell. At present it is necessary to treat plant proteins in groups. The next phase of investigation may reveal how they can be treated as individuals, that is, as specific substances. Ultimately we may hope to learn something of the complexes or systems of inter-reacting substances the behavior of which we recognize as the life process.

The purposes of this review will have been accomplished if better definition has been given to the concept plant protein, and if a little light has been shed upon the position of our present knowledge of these substances. Nothing has been said of their metabolism. Protein synthesis in plants has recently been fully reviewed by the late Professor Petrie (81) of the University of Adelaide, the composition of leaf proteins by Lugg (82), and the virus proteins by Stanley (83), by Bawden (84), and by Hoagland (85). For the most comprehensive discussion of the more general aspects of protein metabolism in plants, reference should be made to Chibnall's Silliman lectures (28). Careful study of all of these papers will show, however, that present knowledge is and will remain largely empirical until the proteins themselves are better understood.

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# PHYSIOLOGICAL REVIEWS

VOL. 25

JULY, 1945

No 3

## THE RELATION OF ADRENALINE TO ACETYLCHOLINE IN THE NERVOUS SYSTEM

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Most people think of adrenaline and acetylcholine as antagonists. The one raises the blood pressure, the other lowers it, the one inhibits intestinal movement, the other increases it, the one dilates the pupil, the other constricts it. Adrenaline produces the effects of sympathetic stimulation, and acetylcholine those of parasympathetic stimulation, and since the sympathetic and parasympathetic systems produce effects almost always in opposite directions, there is abundant reason for the view that adrenaline and acetylcholine have antagonistic actions.

It is now known that not only are parasympathetic effects imitated by acetylcholine, but that acetylcholine is released when an impulse is transmitted from the vagus endings to cardiac tissue (for example), and the transmission of the impulse is due to the acetylcholine. Acetylcholine is also liberated when an impulse is transmitted from somatic motor nerve endings to skeletal muscle, and when an impulse passes from a preganglionic fibre of the sympathetic system to a post-ganglionic fibre. Many believe that at the neuromuscular junction in skeletal muscle and across the sympathetic ganglion, the transmission of the impulse is effected by acetylcholine there too. Finally some evidence has been obtained that acetylcholine is released when impulses cross the synapses of the spinal cord, so that there also, at some synapses, acetylcholine may be the transmitter.

What part, if any, does adrenaline play at these points of transmission of a nervous impulse at which acetylcholine is liberated? Is the transmission of a nervous impulse affected by the presence of adrenaline? Is the effect of acetylcholine, deliberately applied at these points, modified by the presence of adrenaline? These are the main questions now to be discussed.

*Adrenaline action on denervated muscle.* The earliest observations which suggested that adrenaline modifies the action of acetylcholine were made on denervated skeletal muscle. It has long been known that when the postganglionic sympathetic fibres to the pupil of the eye degenerate as a result of extirpating the superior cervical ganglion, the pupil becomes much more sensitive to the action of adrenaline. In the same way, when the motor fibres to skeletal muscle degenerate, the muscle becomes much more sensitive to the action of acetylcholine and of drugs having a similar action, such as nicotine. This increase in sensitivity is very great, and indeed the response of denervated muscle to acetylcholine was known long before its action on normally innervated muscle was discovered, since special methods are needed to display this. Philippeaux and Vulpien (1) showed that when the hypoglossal nerve had been allowed to degen

erate, stimulation of the chorda-lingual nerve, or of the chorda tympani alone caused a contraction of the denervated tongue. Heidenham (2) showed that this denervated tongue also behaved differently from innervated tongue in that it contracted when an intravenous injection of nicotine was given. Frank Nothmann and Hirsch-Kauffmann (3) found that acetylcholine not only acted like nicotine, but did so in very small doses. We see, then, the explanation of the observation of Philippeaux and Vulpian: stimulation of the chorda tympani liberates acetylcholine, and the tongue, sensitized by denervation, contracts in response to it. Other phenomena have received a similar explanation. Sherrington (4) observed that the muscles of the cat's leg and foot when denervated by section of the spinal nerve roots, contracted when the cut end of the sciatic nerve was stimulated six weeks later. The work of Hinsey and Cutting (5) and of Bülbbring and Burn (6) showed that the fibres responsible for the effect were the sympathetic fibres which remained unaffected by the root section, and the last-named authors (7) showed that some of the sympathetic fibres supplying blood vessels in the muscles liberated at their terminations, not adrenaline (or sympathin), but acetylcholine. Here again the explanation of the Sherrington phenomenon is that stimulation of the sympathetic chain liberates acetylcholine at endings in the muscle blood vessels, the muscles of the leg and foot, sensitized by denervation, contract in response to it. (Those wishing to read more of the pharmacology of denervated muscle should consult the paper by Dale and Gasser (8), in which they examined the different substances known to cause contraction, and observed that it was the nicotine-like property of acetylcholine and other quaternary bases which enabled them to act in this way.)

The effect of adrenaline on contractions, or contractures, caused by drugs in denervated muscle has been tested by several workers, but the chief contribution to the evidence was made by Dale and Gaddum (9). They found that if the contraction of the denervated muscle was induced by injection of acetylcholine either intravenously or intra-arterially, then the effect of injecting adrenaline beforehand depended on how long beforehand the injection was made. If the acetylcholine was injected immediately after the injection of adrenaline, then acetylcholine usually failed to produce a contraction at all, on the other hand when the interval between the injections was longer, about one minute, then the effect of the acetylcholine was increased, the contracture being stronger and more persistent. Thus after the injection of adrenaline, there were two phases of the acetylcholine response, an early phase of depression and a later phase of augmentation. The two phases were nearly always observed in the denervated gastrocnemius of the cat, though sometimes the second phase of augmentation was not seen. In the denervated tongue on the other hand the phase of depression was only once seen, and the usual effect was augmentation and, especially, prolongation. Similar results have since been obtained by Luco (10).

Dale and Gaddum then carried out a simple experiment on an isolated strip of denervated diaphragm. It seemed likely that the double effect of adrenaline

might be due to its vascular action complicating another action. To avoid this complication they looked for a skeletal muscle which might be expected to survive for some hours without a circulation provided it was suspended in a well-oxygenated bath of Ringer's solution. They chose the diaphragm of a young cat. One-half of the diaphragm was denervated by removal of a portion of one phrenic nerve, and after 14 days' interval, a strip of the denervated side of the diaphragm was suspended in a bath at 37°C. This strip contracted when 2 microg of acetylcholine was added (to make a concentration of 1 in 7.5 millions). After washing out, the strip relaxed, and it was then observed that if the same dose of acetylcholine was added in the presence of 0.2 mg adrenaline put in the bath 6 minutes before, then the contraction was greater. The bath was again washed out. The acetylcholine contraction became still further augmented when a third dose was added, showing that the effect of the adrenaline persisted for some time after it was removed from the bath, only after two or three washings did this effect disappear. It is important to note that the contraction caused by tetramethylammonium iodide was also augmented by adrenaline as well as that caused by acetylcholine.

This very simple experiment illustrated what was then a quite new and is still a little known aspect of the relation of adrenaline to acetylcholine, in which these substances no longer appear as antagonists but rather as allies, the one potentiating the action of the other. Adrenaline itself is not known to have any action on normal skeletal muscle whether this be frog or mammalian muscle, but there are two observations which have been made concerning its action in denervated mammalian muscle. v Euler and Gaddum (11) observed that adrenaline produced a slow contracture of the denervated canine muscle which runs along the upper lip of the dog. Similarly Bülbring and Burn (6) found that adrenaline produced a slow contracture of the denervated gastrocnemius in both the cat and the dog. The potentiation by adrenaline of the action of acetylcholine is not, however, peculiar to denervated muscle. Bülbring (unpublished observations) has observed that the contraction of the frog's rectus abdominis muscle, suspended in a bath, in response to acetylcholine of concentration  $10^{-7}$  is potentiated by the addition to the bath of adrenaline in a concentration of  $10^{-4}$ . Thus, we see that in skeletal muscle adrenaline potentiates the action of acetylcholine, the action of a substance which is now believed, since the work of Dale, Feldberg and Vogt (12), to be responsible for the transmission of the impulse from the motor nerve ending to the muscle itself.

The fact at once recalls the observations which have been made on the effect of adrenaline in augmenting the contraction of muscle partially fatigued. The earliest observations were made by Gruber (13) who showed that when a rapid series of interrupted tetani were applied to the sciatic nerve of the cat, and when the contractions diminished due to fatigue, the intravenous injection of adrenaline augmented the contractions. Likewise the observations of Orbeli (14) are recalled who showed that stimulation of the motor roots in the frog led to diminishing contractions which were augmented when the sympathetic fibres were stimulated in addition. We know that the sympathetic fibres liberate adrenaline

(or sympathin) and the augmentation described by Orbeli may represent the potentiation by this adrenaline of the acetylcholine liberated at the motor nerve ending. Gruber's observations have been confirmed by many subsequent workers, who have been divided in their opinion whether the action of the adrenaline was exerted at the neuromuscular junction or on the muscle itself. Bülbring and Burn (15) showed that much the greater part of the effect was due to an action at the neuromuscular junction, and so quite possibly related to the release of acetylcholine there.

*Observations in the spinal cord* Between 1930 and 1941 little attention was paid to the observation made by Dale and Gaddum on the strip of cat's denervated diaphragm, it remained as a single observation in a paper full of other observations, and its possible connection with Gruber's work and with the Orbeli phenomenon was not pointed out. In 1941 Bülbring and Burn published observations concerning the transmission of impulses in the spinal cord (16). They made these observations on the lower part of the dog's spinal cord which was perfused with defibrinated blood. They elicited the flexor reflex and the knee jerk in one hind leg, which was perfused by a separate circulation of blood. Their experiments were directed to obtaining evidence that acetylcholine was responsible for the transmission of impulses across the synapses in the cord. Part of the evidence that acetylcholine is a transmitter at the neuromuscular junction is that acetylcholine given in a very small dose by intra-arterial injection at the point where the artery enters the muscle causes a typical muscle contraction (Brown, Dale and Feldberg, 17). In the same way, part of the evidence that acetylcholine may be the transmitter in the sympathetic ganglion is that acetylcholine injected into the perfused superior cervical ganglion causes a discharge along the postganglionic fibres (Feldberg and Gaddum, 18). Bülbring and Burn therefore attempted to demonstrate that when acetylcholine was injected into the blood going to the perfused spinal cord, a discharge of motor impulses from the anterior horn cells occurred, leading to contraction of the anterior tibial muscle in the separately perfused hind-leg. They were at first unable to demonstrate this, they discovered, however, that if adrenaline was present in the blood perfusing the spinal cord, the demonstration was quite easy, and when the acetylcholine was protected on its way through the vessels to the cord by a small dose of eserine given one minute earlier, then 1 microg acetylcholine produced a series of contractions in which tensions more than 10 kgm were developed. The point of interest was the need for the presence of adrenaline in order to demonstrate this action of acetylcholine.

Two other observations were also made. The contraction of the anterior tibial muscle in response to stimulation of the posterior tibial nerve (the flexor reflex) was small unless adrenaline was added to the blood perfusing the spinal cord. Here the normal transmission of an impulse from the sensory to the motor side of a reflex arc was so improved by adrenaline that the tension developed in the contracting muscle rose from 0.3 kgm to 1.3 kgm. The other observation concerned the action of prostigmine. Prostigmine was found to have little or no effect on the flexor reflex. This was a surprising result since when

eserine was injected into the blood going to the spinal cord, the tension developed in the flexor reflex was often doubled. This result conformed with the earlier observations of Merlis and Lawson (19) on the effect of eserine on the flexor reflex. It had been expected that prostigmine would act in the same way. After the addition of adrenaline to the blood, however, the injection of prostigmine did increase the flexor reflex.

In this work on the spinal cord there was evidence that adrenaline augmented both the action of acetylcholine, and also that of prostigmine, since prostigmine by its anticholinesterase action increases the amount of acetylcholine it seemed possible that the synergism between adrenaline and prostigmine was essentially a synergism between adrenaline and the protected acetylcholine, but this will be discussed later.

*Prostigmine in skeletal muscle* The relation found to exist between prostigmine and adrenaline in the spinal cord suggested that a similar relation might exist in skeletal muscle. Bulbring and Burn (20) therefore made experiments in which they recorded the tension developed in the gastrocnemius of the cat when the maximal single shocks were applied to the sciatic nerve every 15 sec. They injected prostigmine into the common iliac artery, and observed that after a dose of 0.02 mgm. a moderate increase in the tension developed at each contraction. When an injection of 0.02 mgm. adrenaline was given four minutes after the injection of prostigmine a further increase of the tension was recorded, about as great as that caused by the prostigmine. In other experiments they injected prostigmine and adrenaline together, comparing the effect with that of prostigmine alone, and found that the combination produced more than double the effect. The action of eserine was also increased by combining it with adrenaline, but the increase was slight, it was very much less than the increase in the effect of prostigmine.

The sciatic nerve was also stimulated at more rapid rates. At these more rapid rates prostigmine caused a greater rise in the tension presumably because acetylcholine accumulated more rapidly, when adrenaline was then injected, the injection no longer caused a further increase, but now depressed the tension for a period. This depressant action was directly connected with the more rapid rate of stimulation for it disappeared when the initial slow rate was resumed. At fast rates of stimulation prostigmine in large dose itself caused depression, and adrenaline intensified this depression. These experiments so far indicated that adrenaline potentiated the effect of prostigmine, converting a small effect into a greater effect, transforming a maximal effect into the effect of excess, namely, a depression, and converting a depression into a still greater depression.

That excess of acetylcholine should depress the effect of nerve stimulation is easy to understand when there is excess the number of enzyme molecules is insufficient to destroy the molecules of acetylcholine present, and many of these remain attached to the receptors on the cell, blocking the access of new molecules. If the size of the muscle response depends on the proportion of acetylcholine molecules released by the nervous impulse which are able to make effective connection with cell receptors, then the response will be depressed if some



receptors are blocked by undestroyed acetylcholine. To explain how adrenaline can potentiate the action of acetylcholine, so that the effect of a small dose is increased, while the effect of a larger dose is decreased, is not so easy. We can suppose that adrenaline increases the permeability of some membrane situated between the point of liberation of acetylcholine and the point of union with the cell receptors. By increasing the permeability, adrenaline increases the proportion of acetylcholine molecules which penetrate the membrane. If the amount of acetylcholine is small, this increase will result in a greater response, if however the amount of acetylcholine is large, the increase will result in a smaller response because there will be an excess of acetylcholine at the point of union with cell receptors.

*Relation of adrenaline to prostigmine* The simplest view of this action of adrenaline is that it is basically the same as the action described by Dale and Gaddum on the denervated muscle of the cat's diaphragm. On the cat's diaphragm in a bath of Ringer's solution, adrenaline potentiates acetylcholine, so that it causes a larger contraction. When prostigmine is injected into the cat, and the sciatic nerve is stimulated, acetylcholine accumulates at the motor end plates, adrenaline injected while the accumulation persists potentiates this acetylcholine and causes a greater contraction.

Against this simple view there are certain arguments to be put forward. If the view were correct, adrenaline should potentiate the action of eserine as effectively as it potentiates the action of prostigmine. There is no doubt at all that it does not do so, either in skeletal muscle or in the spinal cord. That is to say, there is a special relation between adrenaline and prostigmine independent of the action of the latter in preserving acetylcholine from destruction by cholinesterase. Büllbring and Burn (20) obtained the clearest evidence of this on blood vessels. When the vessels of the hind leg of a dog were perfused with defibrinated blood by a pump, the injection into the artery cannula of 4 microg of adrenaline caused the pressure in the cannula to rise and the venous outflow to be reduced. The injection of twice this dose produced a proportionally greater effect. Prostigmine was then injected into the artery cannula in a dose of 20 microg. This injection itself was followed by a moderate but prolonged rise in the arterial resistance, and in addition the action of the doses of adrenaline was increased so that their effect was doubled. After prostigmine, 4 microg of adrenaline had the same effect as 8 microg injected before. Thus the observation of Mendez and Ravdin (21) that prostigmine possesses some peripheral vasoconstrictor action was confirmed and it was also found that prostigmine affects the action of adrenaline on the blood vessels in much the same way as cocaine.

There is an additional reason for supposing that the potentiation of the effect of prostigmine on skeletal muscle by adrenaline is not simply a potentiation of the acetylcholine protected by prostigmine. When the gastrocnemius muscle of the cat is stimulated by an interrupted tetanising current applied to the sciatic nerve 120 times a minute, fatigue ensues, and the tension developed by each short tetanus slowly declines during 2 to 3 minutes and then is maintained

almost steady As was first shown by Gruber (13) the injection of adrenaline at this point increases the tension developed, the increase rising to a maximum and then passing off If, instead of injecting adrenaline, prostigmine is injected the tension at once falls, as was demonstrated by Briscoe (22) It should be noted that this fall occurs not only in fatigued muscle, but also in rapidly stimulated muscle which is not fatigued Thus in muscle subjected to rapid stimulation so that fatigue is produced, adrenaline and prostigmine have opposite effects The former increases the tension while the latter decreases it This is a reversal of the position in muscle stimulated at a moderate rate in which prostigmine causes a large increase in tension, and adrenaline then causes a depression The contrast was found to be still more striking when Bülbring and Burn injected prostigmine into rapidly stimulated fatigued muscle, observing that it caused a decline of the tension, and then injected adrenaline, to find to their surprise that the adrenaline still caused a rise of tension They had assumed that since prostigmine diminished the tension, an excess of acetylcholine must already be present, and expected that the "potentiation" of an excess, if it altered the tension at all, would depress it still more If the dose of prostigmine is large and allowed to act for some time, the injection of adrenaline following prostigmine does in fact cause further depression beyond that produced by prostigmine, but the earlier stage remains to be explained

The conclusion is difficult to avoid that there may be three actions of adrenaline First the action by which the effect of acetylcholine on muscle is intensified, second the action by which the effect of prostigmine is intensified, and third an action now to be discussed When Orbeli (14) first described how stimulation of the sympathetic ramus increased the contraction of the gastrocnemius of the frog, fatigued by repeated stimulation of the motor roots, he explained the effect of sympathetic stimulation as restoring the power to transmit impulses to some of the motor end organs in which as a result of repeated stimulation transmission had failed The idea has generally been expressed by saying that sympathetic stimulation, or adrenaline, "lowers the threshold" for the transmission of impulses at the neuromuscular junction But in what does this lowering of threshold consist? Is it an action at the nerve ending at all? May it not just as well be an effect of sympathetic stimulation or of adrenaline on the transmission of impulses along the nerve?

*Transmission along the course of the nerve* Bülbring and Burn (23) investigated the contractions of the gastrocnemius in response to maximal single shocks applied to the motor roots The lower part of the dog's hindleg was perfused with defibrinated blood, and having prepared the lumbar sympathetic chain for stimulation, they were able to see the effect of the sympathetic on the motor root stimulation The stimuli were applied at intervals of 10 sec so that no question of fatigue at any point could arise It was found that the stimulation of the motor roots became progressively ineffective, the tension developed in the gastrocnemius declining from 9 kgm to zero The tension could be promptly restored either by stimulation of the sympathetic chain for 2 minutes or by the addition of adrenaline to the perfusing blood The restorative effect was at

first thought to be exerted at the neuromuscular junction, because there was no failure of the muscle to contract in response to direct stimulation, and no change in the tension developed by direct stimulation when the sympathetic was stimulated or adrenaline was added. A failure of transmission at the neuromuscular junction in the absence of fatigue had not been previously described, and it was then shown that at a time when stimulation of the motor roots became ineffective, stimuli applied to the sciatic nerve at a point near its entry into the muscle still produced a tension of nearly 6 kgm. That is to say, at a time when stimuli applied to the motor roots was ineffective, the same stimuli applied to the sciatic nerve near the muscle caused the gastrocnemius to contract. Transmission at the neuromuscular junction was still good but transmission along the nerve itself had become defective. It was further shown that application of single shocks to points on the sciatic nerve at increasing distances from the muscle produced contractions of decreasing tension. The injection of adrenaline into the blood, however, increased the distance from the muscle at which a response of given tension could be evoked. These experiments made it clear that sympathetic stimulation, or the presence of adrenaline, exerted a considerable effect on the transmission of impulses along the course of the motor nerves.

The addition of adrenaline to the perfusing blood, or the stimulation of the sympathetic chain of course produced vascular changes in the perfusion system, these were recorded as a rise in the arterial resistance to the inflow of blood and as a diminished venous outflow. It looked at first sight as though transmission along the motor fibres was improved when the blood flow was less. Changes in venous outflow and in arterial resistance, though indicating a total diminution in blood supply, give no indication of what may happen locally, thus it may be that adrenaline causes certain arteriovenous anastomoses to be shut with the result that capillary areas in the neighbourhood obtain a greater supply. It seems very probable that the improved transmission obtained by injecting adrenaline or by sympathetic stimulation was in fact due to vascular changes of the kind indicated rather than to any specific effect of adrenaline itself, since improved transmission along the motor fibres followed the addition to the perfusing blood of pituitary (posterior lobe) extract or of blood which had been standing, in which vasoconstrictor substances had accumulated. On the other hand against this explanation was the rate of recovery. When stimulation was applied to the sympathetic chain, within 30 sec the tension developed in the gastrocnemius in response to single shocks applied to the motor roots had increased from 4 to 6 kgm, and within 60 sec had risen to 9 kgm. Mere improvement in blood flow might be expected to take longer to exert so much effect. Moreover the effect on muscle tension persisted five or six times longer than the effect on blood flow. The fact remains that in the conditions of these experiments, adrenaline, whether injected or liberated at sympathetic nerve endings, was found to affect transmission along motor fibres in a striking manner.

*Adrenaline on action potentials.* That adrenaline affects the action potentials of nerve was later demonstrated by Bulbring and Whitteridge (24). They stimulated the sciatic nerve in the cat, having made a spinal, eviscerated and

adrenalectomised preparation, and recorded action potentials in the posterior tibial nerve. When submaximal stimuli were applied at a frequency of 1 per second, the injection of 5 microg adrenaline caused an increase in the height of response of  $\alpha\beta$  fibres which varied in nerves in good condition from 30 to 115 per cent. In experiments where the nerve had been incompletely guarded from fatigue, increases of 300 per cent in height of response were observed. It should be emphasized that this increase was observed with submaximal stimulation, and not when maximal stimuli were employed.

How is the effect of adrenaline on the transmission of impulses along the course of the sciatic nerve to be explained? Certainly the results of Bülbbring and Whitteridge cannot be attributed to a mere alteration of blood supply. It is true that their best results were obtained in the spinal cat which often has a low blood pressure but they were also obtained in the decerebrate cat or cat anaesthetised with chloralose, and both of these have a high blood pressure. The intravenous injection of 5 microg adrenaline produces a concentration in the blood of a cat weighing 3 kgm roughly equal to  $0.3 \times 10^{-7}$ , and this can have little vascular action.

Recently Nachmansohn has put forward the view that acetylcholine is concerned with the transmission of the nervous impulse along the nerve fibre as well as at the synapse. Fulton and Nachmansohn (25) argue that the main difficulty which has stood in the way of accepting the theory of humoral transmission at nerve endings has been that this theory has implied a basic difference in the method of transmission across a synapse and of transmission along a nerve. They point out that Gasser and Erlanger (26) came to the conclusion that conduction along fibres and across synapses is essentially the same process and that the difference is only quantitative. If however it can be shown that transmission along a nerve involves the activity of acetylcholine, then this difficulty in the way of accepting the theory of humoral transmission disappears. Nachmansohn and Meyerhof (27) have found that in the electric organs of fishes there is a close parallelism between the voltage produced and the concentration of cholinesterase. This suggests a relation between acetylcholine and the electric discharge. More relevant to the present discussion, however, is their further finding of a high concentration of cholinesterase in the sheath of the giant nerve fibre of the squid, the concentration in the axoplasm being negligible. They suggest that the potential differences observed during nerve activity are closely connected with the metabolism of acetylcholine everywhere at or near the surface of the nerve cell (including the axon), and not only at the synapse. If it be true that transmission along nerve fibres involves the release of acetylcholine, then it is possible to relate the beneficial effect of adrenaline on transmission along the sciatic nerve to the other conditions in which adrenaline potentiates the action of acetylcholine. We see again this collaboration which seems to be a fundamental property of the two substances.

*Motor paralysis by sympathetic stimulation.* The parallel does not stop at simple potentiation of nerve transmission by adrenaline. Bülbbring and Burn (23) found that while the ordinary effect of stimulating the sympathetic chain

was to augment the contraction of the dog's gastrocnemius when this was elicited by stimulation of the motor roots, in certain experiments in which a tetanus of 20 sec duration was applied to the roots, stimulation of the sympathetic chain produced, during the stimulation, an abolition of the muscle response followed by augmentation of the muscle response only when the sympathetic stimulation had stopped. Here was seen a paralysis of motor response as a result of sympathetic stimulation. The picture called to mind was that of creatures being "paralysed by fear," so frightened that they are unable to move, being "rooted to the spot." The failure of the motor response in the experiment described must have been due to the liberation of adrenaline, and this when liberated acted in such a way that the transmission of the impulse along the nerve or at the nerve ending by the acetylcholine mechanism was interrupted. We can suppose that the effect was due to an interplay between adrenaline and acetylcholine similar to that observed by Dale and Gaddum (9) when sympathetic stimulation or adrenaline injection abolished the effect of an immediately preceding dose of acetylcholine in denervated muscle.

*Summary of actions in relation to skeletal muscle* It was stated earlier that at least three actions of adrenaline in relation to skeletal muscle must be considered. First the action by which the action of acetylcholine on denervated mammalian or normal frog muscle is intensified, second, the action by which the effect of prostigmine is intensified, and third, the action on neuromuscular transmission which has now been shown to apply to conduction along the nerve fibre also. Orbeli certainly considered that the improvement in the effect of motor root stimulation due to sympathetic stimulation was exerted by lowering the threshold at the neuromuscular junction, the observations of Bülbring and Burn and of Bülbring and Whitteridge clearly concern transmission along the nerve, which adrenaline affects perhaps by modifying the threshold for excitation. It seems probable that it will soon be generally recognised that effects produced on transmission along the nerve cannot be distinguished from similar effects occurring at the neuromuscular junction.

*The sympathetic ganglion* Gaskell, in his book on *The Involuntary Nervous System* (28), points out the close connection between the chromaffine cells which form adrenaline, and the cells of the sympathetic ganglia. "In the adult mammal the chromaffine system is confined to the medulla of the suprarenals, but in the embryo chromaffine cells are found in close connexion with sympathetic cells, a specially large mass known as Zuckerkandl's body which is found at the bifurcation of the abdominal aorta, lasts till birth closely connected with the inferior mesenteric ganglia. As we pass downwards in the animal kingdom, we find the chromaffine cells and sympathetic cells in close contact, even in the adult condition. In the Amphibia, where the sympathetic ganglia are arranged much in the same way as in the Mammalia, the chromaffine cells and the sympathetic cells are mixed close together in every ganglion" (pp 139-140, loc cit). Recently the work of Stöhr (29) has again called attention to the presence of chromaffine tissue surrounding the ganglion cells of sympathetic ganglia.

The first work on humoral transmission in sympathetic ganglia was that of Kibjakow (30) who made a valuable contribution to physiological methods by describing the perfusion of the superior cervical ganglion of the cat with Locke's solution. The perfusion is carried out in a cat in which the normal circulation of the blood is maintained in the rest of the body. In the course of his work Kibjakow observed that if he collected the Locke's solution coming out of the ganglion during a period of prolonged stimulation, and then re-perfused this fluid through the ganglion, the threshold for stimulation was lowered. Using Kibjakow's method, Feldberg and Gaddum (18) demonstrated that stimulation of the preganglionic fibres caused the liberation of acetylcholine from the ganglion, and from then on attention was focussed on acetylcholine as the humoral transmitter.

The first evidence that adrenaline played a physiological rôle in the sympathetic ganglion was that of Marrazzi (31) who published evidence that adrenaline causes depression of the nervous impulse in its passage through the superior cervical ganglion. He made observations in cats and rabbits anaesthetized with pentobarbital, recording the action potentials in the postganglionic fibres when submaximal shocks at a rate of 2 per sec. were applied to the preganglionic trunk. He observed that, when adrenaline was injected intravenously in doses from 5 to 500 microg. the action potentials were reduced. Marrazzi did not observe any stage in which the action potentials were increased and the one effect he recorded was that of ganglionic depression. He also observed that the injection of ephedrine depressed the action potentials in the postganglionic fibres, but the doses he used were very large, being 10 mgm. per kgm. In order to exclude any vasoconstrictor action of adrenaline as the cause of the ganglionic effect, Marrazzi arrested the circulation by piercing the heart while still recording the action potentials. During the first five minutes after death there was no decrease, and indeed there was an increase in the height of the spike which later became greater. It is interesting to note that when Marrazzi tested the effect of larger doses of adrenaline (0.25 mgm.) he observed that in recovery from the initial depression the spike height rose to a slightly higher level than the original.

In 1942 Bülbring and Burn (32) published evidence which indicated that adrenaline exerted two effects on ganglionic transmission, one an augmentor action, and the second a depressor action like that described by Marrazzi. It had been shown earlier, (Burn (33)), that the vasoconstriction produced in a perfused hindleg when the sympathetic chain was stimulated, was greater in the presence of adrenaline. This change was believed to be produced at the postganglionic terminations. The recent paper by Stöhr (29) describing the presence of chromaffine tissue in sympathetic ganglia indicated a different possibility, that adrenaline might improve transmission in the sympathetic ganglia. Bülbring and Burn therefore made experiments in a double perfusion system in the dog. They arranged one circuit of defibrinated blood to perfuse the ganglia of the lateral sympathetic chain from the level of the kidney downwards, and a second circuit to perfuse the corresponding hindleg. They were then

able to test the effect of different concentrations of adrenaline in the ganglion circuit on the effect of stimulating the sympathetic chain. The vasoconstrictor response in the vessels of the hindleg was recorded to measure this effect.

In the leg circuit a steady concentration of adrenaline was maintained throughout the observations. In the absence of any adrenaline from the ganglion circuit, a very small vasoconstrictor response was obtained in the leg vessels when the sympathetic chain was stimulated. When adrenaline was added to the venous reservoir of blood in the ganglion circuit at a rate of 4 microg per min (the total volume of blood in the circuit being 600 cc), then stimulation of the pre-ganglionic fibres gave a greatly increased response. It was concluded that the initial effect of adrenaline on the sympathetic ganglia was to improve the transmission.

A different effect of adrenaline was seen when the amount of it in the blood passing through the ganglia was raised further. When the rate of adding adrenaline to the venous reservoir increased from 4 microg per min to 10 microg per min the response to stimulating the preganglionic fibres diminished, indicating depression of the ganglionic transmission. This second result was in agreement with observations of Marrazzi.

*The pressor action of acetylcholine* In the foregoing experiments the sympathetic ganglia received stimuli from the preganglionic fibres. It is however also possible to stimulate the ganglia by the injection of acetylcholine. To observe this, atropine must be given to exclude the vasodilator and cardio-inhibitory actions of acetylcholine, and the doses employed must be relatively large. Bülbbring and Burn made observations in spinal cats and observed the pressor effect of injecting acetylcholine in doses of 0.4 to 0.6 mgm at regular intervals. They found that when adrenaline in small amount, e.g., 5 microg, was interposed between two acetylcholine injections, the effect of the second injection was often (though not always) augmented.

When however a larger amount of adrenaline was used, 0.03 mgm, the effect of the second injection of acetylcholine was depressed, the depression slowly passing off in the course of 30 min or longer. This depressant action of adrenaline was well shown in experiments on atropinised spinal cats when a slow intravenous infusion of adrenaline was given. The pressor effect of acetylcholine was almost abolished. When the adrenaline infusion was stopped, and the blood pressure, which then fell, was restored to the same height by an intravenous infusion of pituitary (posterior lobe) extract, the pressor effect of acetylcholine was fully displayed. These observations were made in cats from which the suprarenal glands were removed, so that the rise of blood pressure produced by acetylcholine was due to ganglionic stimulation alone without stimulation of the suprarenal medulla. The depressant action of adrenaline resulting in paralysis of the pressor effect of acetylcholine in the atropinised cat has also been observed by Stehle and Melville (34).

*Effect of adrenaline on splanchnic stimulation* The experiments in which acetylcholine was used to stimulate ganglia showed evidence of both effects of adrenaline, the augmentation by small doses and the depression by larger ones.

It was however much easier to observe the depressant effect of the large doses of adrenaline. The reverse was true in experiments in which Bülbring and Burn stimulated both splanchnic nerves in spinal cats from which the suprarenal glands were removed. The stimulation was maximal, and was effected by condenser discharges at rates varying from 8 to 48 per sec. The result of stimulation was recorded as the rise of blood pressure in the carotid artery. Continuous intravenous infusion of adrenaline at rates varying from 4 to 15 microg per min increased the rise of pressure produced by a given stimulation by 70 to 110 per cent. No similar change was produced by continuous intravenous infusion of pituitary (posterior lobe) extract. The increase in the pressor response to splanchnic stimulation during an intravenous infusion of adrenaline might of course have been due to a change in the vessels, it was demonstrated however that there was no corresponding change in the pressor effect of adrenaline injected in a single dose. For example, in one experiment before the intravenous infusion of adrenaline, splanchnic stimulation had a pressor effect little more than half that of 7 microg adrenaline, during adrenaline infusion (2.5 microg per min) the pressor effect of splanchnic stimulation was increased so as to be equivalent to that of 7 microg. The inference was that the increased pressor effect of splanchnic stimulation was not due to a peripheral effect on the vessels, but was consistent with improved ganglionic transmission.

In these experiments adrenaline was infused intravenously at rates as high as 17 microg per min, but at no rate was the effect of splanchnic stimulation depressed. In contrast to intravenous infusion, a single large dose of adrenaline, e.g., from 0.04 mgm. to 0.3 mgm, diminished the pressor effect of splanchnic stimulation for periods varying according to the dose of adrenaline injected. The diminution lasted 30 min after the injection of 0.08 mgm, more than 45 min. after the injection of 0.12 mgm, and over two hours after the injection of 0.3 mgm.

*Results in the perfused ganglion.* The observations so far described were made in circumstances in which it was difficult to be certain that effects following the application of adrenaline were exerted in the ganglion and at no other point. Bülbring (35) has therefore studied the action of adrenaline in the superior cervical ganglion of cat when perfused with Locke's solution by the method first described by Kibjakow (30). The preparation was made in cats with normal circulation except in the perfused ganglion, and since adrenaline was injected into the perfusion system, the effect of the adrenaline was sharply restricted to the ganglion. She observed that when a constant submaximal stimulus was applied at intervals of 2 to 3 min to the preganglionic fibres, the injection of small doses of adrenaline into the perfusion fluid increased the response, which was measured as a contraction of the nictitating membrane. The doses employed were from 0.01 to 0.1 microg. The same increase was seen when adrenaline was added to the perfusion fluid in a concentration of 1 in 100 or 1 in 200 million. The increase was observed only when the stimulation was submaximal and applied at a rate less than 8 per sec. Larger doses of adrenaline



were found to have the opposite effect, and to cause depression of the response. The contractions in response to maximal stimulation were also depressed by these larger doses.

Striking results were obtained when the ganglion was stimulated by injections of acetylcholine. Doses of 10 microg acetylcholine injected into the fluid perfusing the ganglion caused small but regular effects, when an injection of 0.05 microg adrenaline was interposed, there was a very large increase in the effect of acetylcholine, which steadily augmented and then once more declined. Larger doses of adrenaline reduced the effect of acetylcholine. The injection of adrenaline had of course some effect on the rate of flow of the perfusion fluid through the ganglion, but this was brief compared with the effect on the response to preganglionic stimulation or on the response to acetylcholine.

Perhaps the most interesting result obtained by Bülbring was the demonstration that when the preganglionic fibres were stimulated the perfusion fluid leaving the ganglion was found to contain a substance having the properties of adrenaline. Thus the perfusate stimulated the heart of the frog, caused relaxation of the isolated rectum of the pigeon, and gave the fluorescence shown by Gaddum and Schild (36) to be characteristic of adrenaline. When samples of perfusate obtained during stimulation were compared with known adrenaline solutions by these three tests, the perfusate was found to have activity equivalent to the same concentration of adrenaline by each test, and this concentration was actually the concentration which when added to the perfusion fluid augmented the response to preganglionic stimulation.

Thus Bülbring was able to demonstrate that adrenaline could, by an action in the sympathetic ganglion, both augment and depress the effect of preganglionic stimulation, and both augment and depress the action of acetylcholine in the ganglion. Finally she showed that when preganglionic fibres were stimulated, not only acetylcholine, but also adrenaline was liberated in the ganglion, so that the resulting effect in the postganglionic fibre depended on the synergism between the two substances.

*Parasympathetic effects* The demonstration of a synergistic relationship between adrenaline and acetylcholine at different positions in the central and peripheral nervous system, raises the question whether adrenaline affects those peripheral actions of acetylcholine which are readily paralysed by atropine, such as the action on the heart, the blood vessels, the intestines and glands like the salivary glands. So far as the heart is concerned there are no observations which indicate a synergism. On the blood vessels however Katz and Schwartz (37) obtained evidence respecting the influence of adrenaline on the constrictor reaction of the vessels of the rabbit's ear to acetylcholine. The vasoconstrictor action of acetylcholine, which is in some respects similar to that of nicotine, was first observed in the rabbit's ear by Reid Hunt (38). Others who have described similar effects include Fleisch (39), Hirose (40), Feldberg and Minz (41) and Brandt and Katz (42). Katz and Schwartz first observed the reaction of the vessels to acetylcholine, then perfused the ears with adrenaline ( $1$  in  $10^9$  or in

$10^{19}$  for 10 to 20 min, and then washed out the adrenaline with Tyrode's solution. Dilutions of acetylcholine ( $1$  in  $10^4$ ) which when continuously perfused through the ear had previously caused some dilatation, now caused powerful constriction. That is to say, Katz and Schwartz observed that when the vessels had been treated with adrenaline the constrictor action of acetylcholine was greatly enhanced. The dilator action of weaker solutions was converted by adrenaline to a diphasic reaction consisting of dilatation followed by constriction.

Bölbring and Burn (7) observed that the vasodilator action of acetylcholine in the perfused hindleg of the dog was greater when the arterial resistance was maintained at a given point by adrenaline, than when it was maintained at the same point by pituitary (posterior lobe) extract. The same was true for the vasodilator action of histamine, however, so that it was not an effect specific for acetylcholine. Turning to the salivary glands, Stavsky (43) has found that adrenaline, which in a dose of 0.01 mgm does not cause salivation in the cat, greatly increases the secretion caused by pilocarpine, eserine or mecholyl. The action of pilocarpine is not an action exerted through the submaxillary ganglion, and this potentiation of pilocarpine by adrenaline must take place in the gland cells, and not at the synapse of the ganglion.

*The basis of the potentiation.* What is the basis of the potentiation of the action of acetylcholine by adrenaline? The potentiation of the action of acetylcholine by eserine is well known, and depends on competition by eserine molecules for the enzyme cholinesterase. The recent work of Kraymer, Goldstein and Plachte (44) suggests that cholinesterase destroys eserine, though this destruction takes place at a slower rate than that of acetylcholine. Has adrenaline any similar action? Is adrenaline an anticholinesterase? Some action of this kind has been described by Waelsch and Rackow (45) for the oxidation products of adrenaline. They have pointed out that both acetylcholine and adrenaline are substituted methylethanol amines, and that both compounds are N-methylated. Further they say that eserine contains an N-methyl indole portion of the molecule which relates its structure to that of adrenochrome, this being also a substituted N-methyl indole. They investigated the action of oxidation products of adrenaline on the cholinesterase in human serum and found that while the product obtained by oxidation with catechol oxidase was itself inactive, it had a strong inhibiting action when made alkaline. Solutions of adrenaline exposed to air in thin layers possessed increasing inhibiting action.

Ellis (46), however, has found no evidence for inhibition of cholinesterase by adrenochrome using pharmacological methods such as the leech muscle method and the frog's rectus abdominis method. Moreover an explanation based on an anticholinesterase action of adrenaline or its breakdown products would not cover all the facts. In denervated skeletal muscle adrenaline potentiates the action not only of acetylcholine but also of tetramethylammonium iodide. If effects involving prostigmine are also to be fitted into the picture, there must be an explanation of the potentiation by prostigmine of the constrictor action of adrenaline on the blood vessels of the dog. Nor does it seem easy to explain the

potentiation of the effect of prostigmine in skeletal muscle by adrenaline as an additional anticholinesterase action, since the effect of eserine in this tissue is increased very little by adrenaline

The effects of adrenaline may be explained by altered permeability of cell membranes. Acetylcholine molecules before making contact with cell receptors may traverse a membrane first. If adrenaline facilitates this passage, a smaller percentage of molecules of acetylcholine may be destroyed by cholinesterase and a larger percentage may engage with cell receptors. But again this explanation would not cover tetramethylammonium iodide. More facts are needed.

#### DISCUSSION AND SUMMARY

The evidence which has been summarised in the foregoing review indicates that acetylcholine and adrenaline in the nervous system have a different relationship from that existing elsewhere. Whereas in the heart, the viscera and throughout the tissues innervated by the autonomic system these two substances have actions which are antagonistic, in the nervous system on the other hand, adrenaline, having little action of its own, exerts a powerful influence in modifying the action of acetylcholine. In general it can be said that in low concentrations adrenaline augments the effect of acetylcholine, while in higher concentrations it depresses it.

Evidence of this action has been obtained at more than one point of the neurones originating in the anterior horn of the spinal cord. In the presence of adrenaline, acetylcholine injected into the spinal cord causes a motor discharge which probably originates in the anterior horn cell itself. The transmission of impulses along the course of the nerve is greatly affected by adrenaline, or by stimulation of sympathetic fibres. The transmission may be increased as much as two or three times, or it may be depressed. This depression, occurring as it does after sympathetic stimulation, is possibly the physiological change underlying the condition in which an animal is paralysed by fear. Since recent evidence suggests that transmission along the course of nerve fibres involves the liberation of acetylcholine and its destruction by the cholinesterase which is present in the medullary sheath, the effect of adrenaline on transmission may be due to its interaction with acetylcholine in this process also.

At the neuromuscular junction one action of adrenaline has long been known, that in which transmission from the nerve to the muscle is improved by adrenaline or sympathetic stimulation when fatigue has first occurred, this was described for adrenaline by Gruber, and for sympathetic stimulation by Orbeli. A second action has now been demonstrated, namely, the potentiation by adrenaline of the action of prostigmine. It is certainly not easy to ascribe either of these effects to a simple potentiation of acetylcholine by adrenaline, the facts are not so uncomplicated as that, nevertheless a synergism between these two substances is likely to be part of the story.

The picture is most complete for the sympathetic ganglion. Evidence of various kinds indicated that adrenaline in low concentration improved ganglionic transmission and in high concentration depressed it. These indications have

now been superseded by Bülbring's demonstration of the phenomena in the perfused superior cervical ganglion. The transmission of submaximal stimuli applied to the preganglionic fibres is increased by small amounts of adrenaline and depressed by larger amounts. That this effect of adrenaline is due to its potentiation of the acetylcholine transmission is shown by the observation that when the ganglion is stimulated by the injection of acetylcholine into the perfusion fluid, the stimulant action is greatly enhanced by the injection of a small dose of adrenaline and depressed by a large one. Finally Bülbring has shown that adrenaline is liberated from the superior cervical ganglion when the preganglionic fibres are stimulated, so that actually in each ganglion provision is made for adrenaline to exert its potentiating action on the acetylcholine through which the transmission is effected. This strong evidence that such an interplay occurs at all normal times in each sympathetic ganglion is the best reason for expecting a similar interplay at those synapses in the central nervous system where acetylcholine transmits the impulse.

If the action of acetylcholine in the central nervous system, including the brain, is indeed modified by adrenaline, it is conceivable that this modification is the basis of changes in nervous reaction, and even in behaviour, which occur in emotional states when an abnormal concentration of adrenaline is present.

It has also been shown that large doses of adrenaline, injected at a given moment into the general circulation depress ganglionic transmission, so that splanchnic stimulation, for example, is less effective than before. This change, which does not occur during the steady continuous infusion of adrenaline into the blood stream, but only on the administration of single doses, must make the body less capable of maintaining a normal blood pressure, when a sudden stimulus, such as is provided by the crash in an accident, causes the liberation of adrenaline at one moment in the blood stream.

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# THE INTERMEDIARY METABOLISM OF FATTY ACIDS

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**SCOPE OF THE REVIEW** The review is confined to a presentation of the current views on the intermediary metabolism of the fatty acids, i.e., the chemical reactions, so far as they are known, involved in the partial or complete oxidation of fatty acids in mammalian organs. The essence of controversial aspects will be presented in some detail, with an evaluation of the evidence. The more recent papers on the subject will be reviewed against the background of old evidence.

Many subjects collateral to the main topic are excluded. Questions of digestion, absorption, transport and storage of fats are outside the scope of the review (Smith, 159, Bloor, 18, Longenecker, 114). The field of metabolism of phospholipids has recently been well covered (Chaikoff, 32, Sinclair, 158). Special topics such as the metabolism of branched fatty acids have also been recently reviewed (Carter, 30). The main work on the use of deuterium in the study of fat metabolism has been ably presented by Schoenheimer (145, 146, 147). The field of the hormonal regulation of fat metabolism will be discussed only incidentally. There are recent reviews (Ingle, 85, Houssay, 83). Lipotropism has been discussed by Best and Lucas (13) and also Crandall (38). There is also a recent discussion of lipocaine by Dragstedt (52). Ketosis in cattle and the fat metabolism of the mammary gland of the cow is discussed in an interesting series of papers by Shaw and his co-workers (152, 153, 154, 155, 156).

**ENZYMES CONCERNED WITH FAT METABOLISM** The presence of enzymes activating fatty acid molecules or their intermediates in tissues is obvious, but in the main the demonstration of their activity has been in the intact animal or surviving intact tissue. Only a beginning has been made in the study of these enzymes free from cellular structure. In comparison with analogous field of carbohydrate intermediary metabolism, the questions of the types of chemical reactions catalysed, the problem of co-enzymes or other prosthetic groups, the study of possibly associated phosphorylations, etc., is still in its infancy.

Apparently the first enzyme concerned with fat metabolism demonstrated free from cellular structure was beta-hydroxybutyrate dehydrogenase, its action is now fairly well defined. Early observations by Wakeman and Dakin (182) showed that aqueous extracts of dog liver oxidized  $\beta$  hydroxybutyrate to acetoacetic acid, and they subsequently showed (183) that ground liver reduced acetoacetic acid to  $\beta$ -hydroxybutyrate. Wishart (192), using alkaline phosphate extracts, demonstrated by the methylene blue technique the presence of this dehydrogenase in muscle as well as liver. These observations were confirmed by Rosling (144), as well as Thunberg (178). Banga, Laki and Szent-Gyorgyi (4) showed that the co-enzyme necessary for this action was the same as that for the oxidation of lactic acid. Later, Green (74) prepared the dehydrogenase from

pig heart and measured its redox potential. He showed that it is cozymase-I linked and that flavin, flavin-protein or adrenochrome are the necessary carriers for aerobic activity.

Kühnau (102) reported on the activity of an enzyme solution prepared from liver by extraction. He found that prolonged equilibration (20 hrs.) with  $\beta$ -hydroxybutyric acid resulted in a decrease of the substrate greater than that accounted for by the increase of aceto-acetic acid. By qualitative tests he reported the formation of aldol, 1,3, butyleneglycol, acetaldehyde, succinic, fumaric and malic acids as well as traces of acetic and pyruvic acid. These results lack confirmation and must be regarded with some reserve. Leloir and Muñoz (110) have prepared from liver by homogenization an enzyme which oxidizes butyric acid. The preparation is done under a continuous stream of oxygen, and at 0°C. to avoid over-heating, since the enzyme is otherwise rapidly inactivated. The resultant cell-free preparation rapidly oxidizes (25°C.) butyric acid. Succinic, fumaric and citric acid added to the system increased the rate of disappearance of the butyric acid, particularly if the preparation had been inactivated by anaerobiosis during preparation. In a subsequent paper Muñoz and Leloir (135) described their experiments in more detail. Preparations partially inactivated by anaerobiosis are restored by the addition of heated liver extract, indicating the necessity for a co-enzyme. For full activity the presence of phosphate, fumarate, cytochrome C, magnesium (or manganese) and adenylic acid were necessary. Of a large number of substrates tested only butyrate, valerate, hexanoate, heptanoate, octanoate were appreciably oxidized. No significant activity was found with formate, acetate, lactate, pyruvate, propionate. The higher fatty acids brought about a decrease in oxygen uptake. Coupling with phosphorylations seems indicated since phosphate was necessary for full activity, and the inhibitors of phosphorylations (fluoride, iodoacetate) inhibited the oxidation. This system does not appear to be identical with that of Lang (*infra*) since it is unstable, and does not oxidize the higher fatty acids. The concomitant oxidation of fumarate was required to obtain maximum activity of the enzyme, indicating a possible involvement of the Krebs cycle. The precise product of oxidation remains to be determined. The authors surmised it to be  $\beta$ -hydroxybutyric acid (no aceto-acetic acid is formed) since approximately one molecule of oxygen is consumed per mole of butyric acid decrease.

Lang, in a series of papers (103, 104, 105, 106), described an enzyme obtained from rat liver by extraction with phosphate at pH 8. It acted as a dehydrogenase upon either odd or even fatty acids, activity increasing with the length of the acid chain. The system had little effect on unsaturated fatty acids and appears to be specific for the saturated higher ones. The products of oxidation were shown to be unsaturated fatty acids. A co-enzyme, which may be obtained from heated rabbit muscle extracts, appears necessary. This was shown to be muscle adenylic acid, that from yeast being inactive except at higher concentrations. Inosinic acid, adenosine triphosphate, and muscle adenosine in equimolar amounts are equally effective. Adenine is ineffective. The

product of the action of the enzyme upon stearic acid was shown to be oleic acid and not an alpha beta unsaturated octodecenoic acid

Fontaine (66) has confirmed Lang's findings of a fatty acid dehydrogenase in liver which requires the presence of a coenzyme for activity Quagliariello (139) reported the presence in bile of a dehydrogenase which is active in the presence of stearic acid causing an extra consumption of oxygen A double bond is formed The activity of the enzyme was inhibited by KCN, while carbon monoxide was without influence Mazza and Stolfi (123) also studied this system and demonstrated the presence in liver extracts of an enzyme which oxidizes stearate in the presence of oxygen They were unable to show its presence in muscles, kidneys, or pancreas In further studies Mazza and Marfori (122) showed that the system brings about oxygen uptake, an action which is inhibited by carbon monoxide, indicating a possible involvement of the cytochrome system Diphosphorase, glutathione, diphosphopyridine nucleotide, and adenylic acid were without influence Since the system was sensitive to cyanide the authors concluded that it contains a heavy metal The action was much more rapid upon fatty acids saturated or unsaturated, containing 12 or more carbons The authors made the interesting observation by means of spectroscopic studies that the oxidation was in the alpha beta position Quagliariello (140) working with the same system reported that stearic and oleic acids were oxidized with equal rapidity

Behrend (11) by glycerol extraction of pancreas obtained an enzyme system which acted upon tristearin with the formation of double bonds Adipose tissue itself has been shown to contain fatty acid dehydrogenases by Shapiro and Wertheimer (151) who extracted one which depended for its activity upon adenylic acid and inorganic phosphate The optimum pH was 8, and the action was greatest with the natural long chain acids, decreasing significantly with diminishing chain length Neutral fat was not acted upon, but phospholipids were Of the non fat substrates only succinate was oxidized with rapidity comparable to that with palmitic and stearic acids The enzyme, or a similar one, was also demonstrated in liver, muscle, heart, and testes Iodo-acetate, fluoride, malic acid, pyrophosphate and benzoate had no effect, whereas exposure to oxygen appears to inhibit the enzyme activity Annau, Eperjessy, and Felszeghy (1) also described a fatty acid dehydrogenase which they obtained from beef liver This, as demonstrated by the methylene blue technic, oxidized lecithin, stearic and palmitic acids An activator—which was obtained from boiled liver extracts—appeared necessary for full activity Extended work on isolation, purification and identification showed that the activator was mainly hypoxanthine In confirmation, xanthine and hypoxanthine were found to be effective as activators Apparently the enzyme system does not oxidize xanthine for the authors state that "das Enzyme ist nicht Xanthineoxidase" This unusual rôle of purines as co-enzymes is not elaborated upon, and the possibility that hydrogen peroxide formed upon oxidation of xanthine produces secondary oxidation effects upon the fatty acids was not discussed Presumably this possibility was excluded by the authors



Lehninger (107) made an attempt to obtain by extraction from rabbit kidney and muscle an enzyme system free from cells and active in the oxidation of acetoacetate. Apparently feeble but definite activity was found in his preparations. Acetoacetate disappeared, the end products not being identified except that approximately 25 per cent of the acetoacetate was recovered as acetic acid. The enzyme was unstable being inactivated in 1 hour at 38°C, hence further purification was difficult. Although not established, a cofactor appears necessary.

The information concerning these enzymes is summarized in table 1.

TABLE 1  
*Cell free enzyme systems concerned with fat metabolism*

ORGAN	SUBSTRATE	PRODUCT OF OXIDATION	COENZYME OF ACTIVATOR	REFERENCE
Liver muscle	$\beta$ -hydroxybutyrate	Acetoacetate	Cozymase I	Wakeman and Dakin, 182-3, Green, 74
Liver	$\beta$ -hydroxybutyrate	Acetoacetate misc oxid products	Not stated	Kühnau, 102
Liver	Lower fatty acids	$\beta$ hydroxy- butyrate	C <sub>4</sub> dicarboxylic acids, adenylic acid phosphate Mg (Mn)	Leloir and Muñoz, 110, 135
Liver	Higher saturated fatty acids	Unsaturated acids	Adenylic acid	Lang, 103-106
Bile	Stearic, oleic acids	Unsaturated acids	Not stated	Quagliariello, 139
Liver	Palmitic, stearic acids, lecithin	Reduction products	Hypoxanthine	Annau et al , 1, 2
Kidney muscle	Acetoacetate	Not known	Not stated	Lehninger, 107

It is too early to evaluate the significance of these studies on isolated fatty acid oxidizing enzymes. Aside from the well worked out case of  $\beta$ -hydroxybutyrate dehydrogenase, the evidence is conflicting. All of the preparations used were impure, and in most cases a co-enzyme appears to be necessary for activity. At least one system was shown to be concerned with the oxidation of the low molecular weight acids with the production of  $\beta$ -hydroxybutyrate. This was found in the liver only, whereas the others were more widespread and were active upon the higher acids, the product of oxidation being a desaturated acid. In some cases adenylic acid appeared to be the co-enzyme whereas in others no co-enzyme has been demonstrated.

Nevertheless, an encouraging beginning has been made upon a subject which will require enormous labor before any clarifying generalizations can be made.

**KETONE BODY PRODUCTION** It is becoming increasingly clear that the formation of ketone bodies (acetoacetate and  $\beta$  hydroxybutyrate) in the liver is an important initiating step in the catabolism of fatty acids for the energy requirements of the peripheral tissues

**Site of formation** It is generally agreed that the liver is the sole site of ketone formation Attempts to show extra hepatic ketone formation have failed Eviscerated preparations from fasted or diabetic animals show a steady fall of blood ketone body concentrations Mirsky (129) showed that anterior pituitary extracts which were actively ketogenic in the intact animal had no effect upon blood ketones in the eviscerated preparation Harrison and Long (78) found, in severe ketotic states, no ketones whatever in muscle water even in the presence of high blood ketones, indicating a complete oxidation rather than a production of ketone bodies at this site.

However, Jowett and Quastel (89) found a small production of ketone bodies using kidney, spleen and testis in vitro

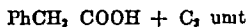
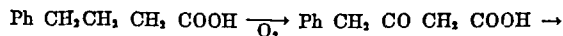
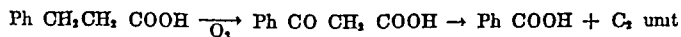
**Precursors** It has long been recognized that the chief formation of the ketone bodies is by partial oxidation of the fatty acids in the liver Certain of the amino acids are known to be ketogenic and they are listed by Shaffer (149) as follows leucine, tyrosine and phenyl alanine Isoleucine (Butts, Blunden and Dunn, 26) also is ketogenic However, the amounts derived from protein are relatively small Carbohydrate or any of its intermediates as a possible source of the ketone bodies is generally excluded Some caution must be exercised on this point, however, in view of Krebs' (97) evidence from experiments with liver in vitro that acetoacetate may arise from pyruvate according to the reaction

$2 \text{ Pyruvic acid} + \text{O}_2 = \text{Acetoacetic acid} + 2\text{CO}_2 + \text{H}_2\text{O}$  Whether this pathway from carbohydrate to ketone bodies plays any significant rôle in the problem of ketosis is undetermined

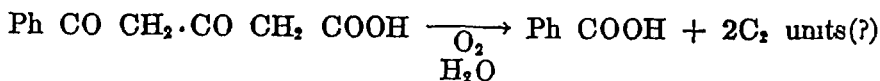
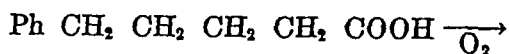
**CHEMICAL MECHANISM OF KETONE PRODUCTION FROM FATTY ACIDS** Three theories have been presented to explain the production of ketone bodies by partial oxidation of fatty acids in the liver Viz

- 1 Successive beta oxidation
- 2 Multiple alternate oxidation
- 3 Successive beta oxidation + condensation

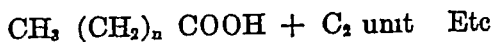
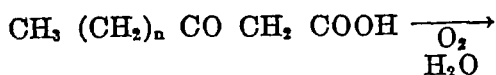
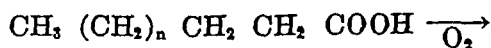
1 *Successive beta oxidation* The original experimental work of Knoop (92) upon which the theory was based was confined to the study of phenyl-substituted fatty acids in which the side chain contained 5 carbons or less When these acids were fed, the nature of the phenyl residue excreted clearly showed that these acids were oxidized at the beta carbon, viz



The  $\beta$  keto acids with 3 or 4 carbons in the side chain initially formed were presumably further degraded by the splitting off of a two carbon unit, the fate of which was never determined satisfactorily. The phenyl residue, either benzoic or phenyl acetic acid, underwent a secondary reaction with glycine forming hippuric or phenaceturic acid. But in the case of valeric acid, the longest fatty acid which he studied, Knoop concluded that there was both beta and delta oxidation, viz



He made no assumptions concerning the possible splitting off of a two carbon unit or whether the oxidations were successive or simultaneous. This classical work has been amply confirmed, and there can be no doubt that beta oxidation not only of short fatty acids which may be used experimentally, but also of the naturally occurring long fatty acids, occurs in the body. For example, Stetten and Schoenheimer (172) using deuterium showed that stearic acid can be degraded to a small extent to palmitic, lauric and myristic acids. The recent work of Bloch and Rittenberg (17) (v. infra) on the acetylation of foreign amines may also be mentioned. The beta oxidation hypothesis has continued to stand upon firm ground, but difficulties arose when a specific application of it was developed which sought to explain the entire catabolism of fatty acids. Dakin (45) from his experiments became convinced that the acid  $\text{C}_6\text{H}_5\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-COOH}$  "undergoes oxidation in the body in such a way that four carbons are removed from the side chain in two pairs." This process "may be termed successive beta oxidation" and he saw "no reason to suppose that it is not a general biochemical reaction" and "that the catabolism of a fatty acid group  $\text{CH}_3\text{-(CH}_2)_n\text{-COOH}$ , is effected by the successive removal of two carbon groups at a time." The successive beta oxidation hypothesis came to mean that each molecule of fatty acid is oxidized through a succession of fatty acids each shorter by two carbon atoms than its immediate precursor, viz

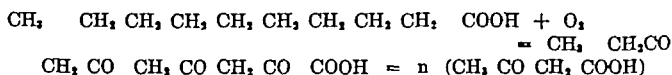


Finally, one molecule of butyric acid remains which in turn is oxidized to acetoacetic acid. In other words, each fatty acid molecule yields only one molecule of acetoacetic acid, the balance being converted into two carbon compounds presumably acetic acid.

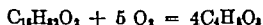
This theory despite its patent defects remained in almost unchallenged ascendancy for a period of forty years, and the reasons are not far to seek. 1, when com-

bined with the theory of obligatory ketone body-carbohydrate oxidation (q v) it explained the ketonuria in diabetes and the marked recession of ketosis following the resumption of carbohydrate oxidation, 2, it permitted the under utilization school of diabetic students to explain how the complete diabetic, who was losing about 80 per cent of the energy from protein as carbohydrate or ketone bodies, and 100 per cent of that from carbohydrates, could still exist. For there was still available the energy derivable from the hypothetical two carbon unit, presumably acetic acid, split off from fats.

**Multiple alternate oxidation.** But the weaknesses of the successive beta oxidation theory which were apparent on closer inspection eventually brought about its dissolution. Though vigorously sought for, the hypothetical acetic acid formed was never demonstrated (Embden and Michaud, 63, Toenniesen and Brinkman, 179, Stadie, 169). Furthermore, the hypothetical intermediate acids with less than 12 carbon atoms were never found in tissues or fat depots of the mammalian organism (36). Small amounts of myristic and lauric were found but none with fewer carbon atoms. On the basis of these and other considerations, Hurlley (84) and later Jowett and Quastel (88) proposed the multiple alternate oxidation hypothesis. According to this the fatty acids are oxidized simultaneously along the whole length of the fatty acid chains at alternate carbons with the result that the entire molecule is converted into ketones, viz



A typical fatty acid such as palmitic would thus yield 4 ketone molecules rather than 1. Viz



The evidence supporting this hypothesis accumulated. Liver slices when equilibrated in the presence of various fatty acids ( $C_4$ - $C_{10}$ ) were found to give higher amounts of ketone bodies in comparison to that formed from the lower ones, a fact which could be explained only when it was assumed that more than one ketone molecule was derived from each fatty acid molecule (Jowett and Quastel, 88). Leloir and Muñoz (110) also studied ketone formation by liver slices in the presence of added fatty acids. Octanoic acid as compared to butyric gave amounts of ketone formation in excess of the 1:1 ratio. On feeding salts or ethyl esters of fatty acids up to  $C_{18}$  to fasting rats, Deuel and his co-workers (27, 47) found so much higher relative rates of ketone excretion from the higher fatty acids that it appeared unlikely that the formation of only one mole of ketones per mole of fatty acids would account for their findings. Blixenkron, Moeller (14) determined the oxygen uptake and ketone production of perfused diabetic cat livers. He found a low  $O_2$  to ketone ratio and he concluded that practically the entire molecule of fatty acid was converted to ketone bodies without the production of any two carbon residue such as acetic acid. Stadie (166), using liver slices from diabetic cats, found the values: oxygen consumption

to ketone formation given in the table 2. When the total oxygen uptake was corrected for carbon dioxide formation and for the observed deamination of amino acids, the observed ratio oxygen ketone formation did not differ significantly from that required by the multiple alternate oxidation hypothesis. In later work Stadie and his co-workers (169) by a method capable of detecting very small amounts of acetic acid were unable to find a trace of acetic acid formation by liver slices which were actively producing ketone bodies. In addition, the formation of any hypothetical intermediary acids ( $C_4$  to  $C_{10}$ ) was excluded. Further, balance studies on fatty acid decrease compared to ketone body increase showed that all of the former would be accounted for by ketone formation, a finding in accordance with the multiple alternate oxidation theory.

*Beta oxidation-condensation theory* The summation of this evidence appears to be convincing proof that the major portion of fatty acid oxidation in the liver,

TABLE 2

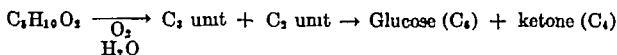
*The oxidative metabolism of liver slices from six depancreatized cats (Stadie, 166)*

OXIDATIVE PROCESS	MEAN OXYGEN UPTAKE MICROMOLES/GM /HR.
Deamination of amino acids calculated from urea and $NH_3$ formation	8.2 $\pm$ 1.2
Carbon dioxide formation	28.0 $\pm$ 5.0
All other oxidations, calc. by difference	51.3 $\pm$ 6.5
Observed oxygen uptake	87.5 $\pm$ 4.0
Ketone formation, observed	46.5 $\pm$ 4.0
Corrected oxygen to ketone ratio observed	1.10 $\pm$ 0.12
Calculated ratio for palmitic acid	
Multiple alternate oxidation	1.25
Successive beta oxidation	6.5

at least that concerned with ketone production, occurs in such a way that the initiation of oxidation upon a given molecule proceeds to completion with great rapidity so that the entire molecule is oxidized to ketone bodies. No intermediary fatty acids are formed nor does any 2 carbon unit accumulate. This is not to imply that beta oxidation, in the original meaning of Knoop, does not occur, for the recent work of Stetten and Schoenheimer (172) proves that a minimal amount of step-wise degradation occurs. Nor does it exclude the possibility that fatty acids may, to a certain extent, yield acetic acid, as the work of Bloch and Rittenberg (17, *infra*) has amply shown. But the implication of the successive beta oxidation hypothesis as later developed and so firmly entrenched in biochemical thinking that large amounts of intermediary fatty acids down to butyric acid are formed in the liver together with acetic acid available as a peripheral fuel can no longer be held.

The original postulation of the multiple alternate oxidation hypothesis assumed

no detailed mechanisms by which the oxidation was brought about. However, mechanisms became read into the original hypothesis and it was implied by many that long chain polyketo acids were formed which were then split into four carbon units which then formed ketone bodies. But certain findings were incompatible with this conception. How could fatty acids with carbons not an even multiple of 4 be entirely converted into ketones? Only by assuming that the extra  $C_2$  units were converted to ketones. In particular, caproic acid ( $C_6$ ) was found to yield a greater amount of ketones than butyric acid (Deuel et al., 47). Caprylic acid is known to be glucogenic and to a certain extent ketogenic (Jowett and Quastel, 88) (Leloir and Muñoz, 110). If these transformations occur simultaneously some such reaction as



would again imply the formation of ketone bodies from a two carbon unit. MacKay (115) proposed that these difficulties of the multiple alternate oxidation hypothesis be resolved by assuming the following mechanism: by successive beta oxidation the fatty acid molecule is split into 2-carbon units. But whatever their nature, these are re-assembled *in statu nascendi* into ketone bodies. The essential implication of the multiple alternate oxidation hypothesis is retained, that is, whenever oxidation is initiated on a given fatty acid molecule it proceeds practically instantaneously at alternate carbons along the entire chain to complete disruption into ketones somewhat like the successive explosions of a pack of fire-crackers. The sole difference between the two hypotheses is that fatty acids are split, on the one hand, into four carbon units forming ketone bodies, and, on the other, into two carbon units which are at once reassembled into ketones. The physiological significance of the two hypotheses is the same, but widely at variance with the successive beta oxidation hypothesis.

This proposed mechanism became known as the beta-oxidation-condensation hypothesis. MacKay (115) urged that the well known conversion of acetic acid to ketone bodies was proof for the beta-oxidation-condensation hypothesis, but this fact has been just as eloquently used by Friedemann (69 *infra*) to confute the original application of beta oxidation to fatty acid metabolism.

Direct proof came from the experiments of Weinhouse, Medes and Floyd (187). They equilibrated liver slices of rats with octanoic acid containing heavy carbon in the carboxyl group. The acetoacetic acid formed was broken down into acetone and  $CO_2$ , and the heavy carbon in these fractions was determined. Their results are shown in table 3. The observed and calculated values for beta oxidation-condensation agreed, and the authors concluded that in the liver oxidation of octanoic acid proceeds by mechanism involving splitting into 2 carbon fragments which condense to the ketone bodies. These interesting experiments give strong support to the beta oxidation-condensation hypothesis.

To emphasize the difficulty of determining the fine details of the mechanism of fatty acid oxidation, it must be remembered that Weinhouse's experiments

could just as well be explained by another hypothesis. If there exists an equilibrium between a two-carbon unit and acetoacetate, e g.,



an assumption supported by the well-known fact that acetic acid is easily converted to ketone bodies (Swenseid, 175), then a random distribution of the heavy carbon in the carbonyl and carboxyl groups would occur irrespective of the mechanism of oxidation of the original octanoic acid molecule.

To emphasize further the difficulties involved, the experiments of Morehouse and Deuel (133) must be discussed. After feeding  $\alpha$ - $\beta$  dideuterio caproate to

TABLE 3

*Comparison of observed values of  $C_{13}$  distribution in acetoacetic acid predicted by various theories of ketone body formation (Weinhouse, Medes and Floyd, 187)*

	ATOM PER CENT $C^{13}$ EXCESS	
	Carbonyl group	Carboxyl group
Observed	0.84	0.85
Calculated		
Beta oxidation-condensation	1.10	1.10
Multiple alternate oxidation	0.00	2.20
Successive beta-oxidation	0.00	0.00

rats they obtained 6.4 atom per cent of deuterium in the excreted  $\beta$ -hydroxybutyric acid, whereas with  $\beta$ - $\gamma$  di-deuterio caproate they found 10.9 atom per cent. The following schema illustrates the possibilities involved.

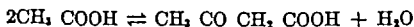
	Intermediates	Possible deuterio $\beta$ -hydroxybutyric acid	Expectancy in deuterio $\beta$ -hydroxybutyric acid
Beta oxidation			
$-\text{C}-\text{C}-\text{C}-\text{CD}-\text{CD}-\text{CO}$	$-\text{C}-\text{CO}-\text{C}-\text{CO}-$ $-\text{CD}-\text{CO}$		None
$-\text{C}-\text{C}-\text{CD}-\text{CD}-\text{C}-\text{CO}$	$-\text{C}-\text{CO}-\text{Cd}-\text{CO}$ $-\text{C}-\text{CO}$	$-\text{C}-\text{CO}-\text{Cd}-\text{CO}-$	Trace
Beta oxidation-condensation			
$-\text{C}-\text{C}-\text{C}-\text{CD}-\text{CD}-\text{CO}$	$-\text{C}-\text{CO}-$ $-\text{CD}-\text{CO}-$	$-\text{CD}-\text{CO}-\text{Cd}-\text{CO}$	Equal
$-\text{C}-\text{C}-\text{CD}-\text{CD}-\text{C}-\text{CO}$	$-\text{C}-\text{CO}-$ $-\text{CD}-\text{CO}$	$-\text{CD}-\text{CO}-\text{Cd}-\text{CO}$	
Beta-delta oxidation			
$-\text{C}-\text{C}-\text{C}-\text{CD}-\text{CD}-\text{CO}$	$-\text{C}-\text{CO}-$ $-\text{C}-\text{CO}-\text{Cd}-\text{CO}-$	$-\text{C}-\text{CO}-\text{Cd}-\text{C}-$	Trace
$-\text{C}-\text{C}-\text{CD}-\text{CD}-\text{C}-\text{CO}$	$-\text{C}-\text{CO}-$ $-\text{CD}-\text{CO}-\text{C}-\text{CO}-$	$-\text{CD}-\text{CO}-\text{C}-\text{CO}-$	Greater

According to Morehouse (132) only traces of deuterium are retained in deuterio  $\beta$ -hydroxybutyric acid when it is in the alpha position, accordingly deuterium in this position is indicated by "d". The experimental findings are not in complete accord with any of the hypotheses illustrated. Beta oxidation appears definitely ruled out. Beta oxidation-condensation requires equal quantities of retained deuterium in the beta hydroxybutyric acid forming from the two caproates, contrary to the findings. The results are in best accord with beta-delta oxidation. This would mean that during the oxidation of the caproic acid a four carbon unit has retained its integrity, a conception implicit in the multiple alternate oxidation hypothesis. To explain the finding of finite amounts of deuterio-beta hydroxybutyric acid from alpha beta di-deuteric caproic acid, it is conceivable that beta-oxidation-condensation and multiple alternate oxidation occur simultaneously, the latter predominating.

Another difficulty might be mentioned. Bloch and Rattenberg (17) found no significant labelled acetylation following ingestion of 9 to 10 di-deuteric stearic acid, a result which they attributed to deposition rather than catabolism of the labelled fatty acid. However, it is also possible that with the higher fatty acids beta oxidation is minimal, a supposition in accord with the experiments of Stetten and Schoenheimer already quoted.

In summary it may be concluded that beta oxidation in the strict sense initially defined by Knoop, is a real phenomenon, that the special application to explain the entire oxidative catabolism of fatty acids generally known as the successive beta oxidation hypothesis must be discarded, that ketone bodies are formed in the liver by conversion of the entire molecule of fatty acid. The evidence is good that two carbon units are formed as intermediate which become stabilized as ketones, in acetyl groups, in cholesterol, or other undetermined forms. But in some cases four carbon units also form. Hence, at the present moment, it is impossible to generalize as to whether one or the other mechanism is the exclusive or predominating one.

*Ketone formation from acetic acid* In discussion of theories of ketone production acetic acid occupies a prominent place. The original investigation of Loeb (113) and Friedemann (69) by liver perfusion experiments showed that the addition of acetic acid was followed by abundant ketone formation. The supposition is that it is converted to ketone bodies presumably by the reaction



Acetic acid is also converted to ketones by liver slices (88, 111), and when fed to the intact animal ketone body production is increased (116) as determined by urine and blood measurements. By the use of acetic acid containing heavy carbon in the carboxyl group, it has been shown by Swensid, Barnes, Hemingway and Nier (175) that the production of ketones may, in part at least, be due to condensation. However, analysis of their data shows that a relatively small proportion of ketone bodies produced originated from the heavy carbon acetic acid fed. The balance must have been formed by some other mechanism initiated by the ingestion of the acetic acid.



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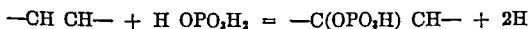
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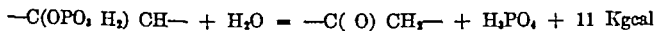
	Intermediates	Possible deuterio $\beta$ hydroxybutyric acid	Expectancy in deuterio $\beta$ hydroxybutyric acid
Beta oxidation			
—C—C—C—CD—CD—CO	—C—CO—C—CO— —CD—CO		None
—C—C—CD—CD—C—CO	—C—CO—Cd—CO —C—CO	—C—CO—Cd—CO—	Trace
Beta oxidation-condensation			
—C—C—C—CD—CD—CO	—C—CO— —CD—CO—	—CD—CO—Cd—CO—	Equal
—C—C—CD—CD—C—CO	—C—CO— —CD—CO	—CD—CO—Cd—CO—	
Beta-delta oxidation			
—C—C—C—CD—CD—CO	—C—CO— —C—CO—Cd—CO—	—C—CO—Cd—C—	Trace
—C—C—CD—CD—C—CO	—C—CO— —CD—CO—C—CO—	—CD—CO—C—CO—	Greater

pathway to carbohydrate. Caprylic acid, on the other hand, yielded ketone bodies only. He assumed that part of the oxidation of butyric acid is by gamma oxidation to succinic acid which is then transformed to glucose. Kleinzeller (91) studied the metabolism of a large number of four carbon monocarboxylic acids. Of these butyric, crotonic, vinylacetic,  $\beta$  hydroxybutyric,  $\gamma$  hydroxybutyric, dl  $\alpha$   $\gamma$ -dihydroxybutyric were readily oxidized by kidney slices. Vinylglycollic,  $\alpha$ -hydroxybutyric, tetrolic, dl- $\beta$   $\gamma$  dihydroxybutyric and tetrionic acids were not appreciably oxidized. He concluded from his data on rates of oxidation that  $\beta$  hydroxybutyric acid is not an intermediary in the oxidation of butyric acid in the kidney, thus indicating that the metabolic pathway in this organ is different from that in the liver where, as is well known, butyric acid readily yields  $\beta$ -hydroxybutyric acid. Vinyl acetic acid ( $\text{CH}_2=\text{CH}-\text{COOH}$ ) was found to be metabolically very active and similar to butyric acid in its reactions, a fact leading Kleinzeller to suggest that it is an intermediate in the metabolism of butyric acid. He further confirmed Jowett and Quastel (88) in finding that malonate inhibited the oxidation of all active substrates enumerated.

Lipmann and Perlman (112) confirmed Quastel's opinion that  $\beta$ -hydroxybutyrate does not form from crotonic acid by the addition of the elements of water in spite of the existence of fumarase or aconitase in tissues which brings about the transformation of unsaturated polycarboxylic acid to the corresponding hydroxy acids. For they were unable to show any formation by liver slices of  $\beta$  hydroxybutyrate from crotonate anaerobically.  $\beta$ -hydroxybutyrate is the reduction product, not the precursor of acetoacetate. In discussing desaturation as an intermediary step in fat oxidation they suggested that desaturation may be associated with phosphorylation, viz



Through this reaction an energy rich phosphate bond is formed which can be utilized in the final reaction leading to the formation of a keto compound viz



Further work on the possible phosphorylation of fatty acids during oxidation was reported by Lehninger (110). Using rat liver homogenates he found that there was a considerable extra oxygen uptake with normal saturated fatty acids having 4 to 18 carbons when cytochrome C and adenosine polyphosphate were present. His experiments suggested that there is an intermediate formation of acyl phosphate because synthetic acyl phosphates (C 8-10) apparently did not require ATP for oxidation.

Cohen (35) studied ketone formation by rat liver slices. He used a large variety of fatty acids and their homologues, viz normal fatty acids ( $\text{C}_2$  to  $\text{C}_8$ ), amino acids,  $\alpha$  hydroxy and  $\alpha$  keto acids ( $\text{C}_4$ ,  $\text{C}_5$ ,  $\text{C}_6$ ), many types of 4, 5, and 6-carbon fatty acids. In an interesting discussion he pointed out that a definite chemical grouping is necessary for oxidation by what he calls the beta-oxidase system. In skeleton form the specific group has the structure  $-\text{CH}=\text{CH}-\text{CO}-$ . On the basis of this hypothesis he explains the results obtained with his divergent



to be accompanied by an initial drop in blood ketones followed in 2 to 3 hours by a high level indicating a quicker response in this species

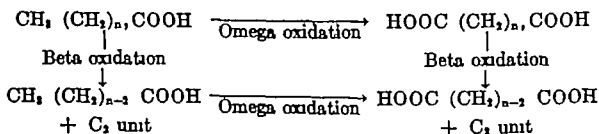
This question of lag becomes important in the problem of the mode of oxidation of fats by muscle (q v)

*Miscellaneous Factors Endocrine relations* Mirsky (126) reported the interesting observation that the ketonemia regularly following the injection of anterior pituitary extract was suppressed in rabbits by the simultaneous administration of insulin. This effect was attributed to the specific action of insulin upon the liver in preventing hepatic glycogenolysis

*Acidosis and alkalosis* The well known ketogenic effect of alkalosis has been studied by MacKay, Wick, Carne and Barnum (120). Using the blood level of ketones in fasting rats as the criterion, they found that acid administration increased liver glycogen, daily excretion of nitrogen and sulphur, while alkalosis leads to opposite findings. They concluded that the corresponding increased or decreased glucose formation from protein explains the resulting effects upon blood ketones

*Ketone formation and ammonia* The well known effect of increased ketone production by liver slices in the presence of ammonia (56) continues unexplained. Linoleic acid was reported to decrease the ketogenic action of ammonium salts (Annau, Eperjessy and Zathureczky, 2)

*Omega oxidation and the catabolism of dicarboxylic acids* The idea that the fatty acids are catabolized by way of a preliminary oxidation of the terminal methyl group with the production of dicarboxylic acids as intermediaries has not escaped advocacy. The chief exponent of this theory of omega oxidation is Verkade (180), who has set forth his views in detail in the reference cited. Following the accidental discovery of diaciduria upon the administration in man of tridecylin ( $C_{13}$ ), he elaborated the theory illustrated by the following schema



Monocarboxylic fatty acids are oxidized on the terminal methyl group with the formation of a dicarboxylic acid. Subsequently by bilateral beta oxidation the dicarboxylic acid is degraded two carbon units at a time with the formation of acetic acid and a dicarboxylic acid containing two carbons less than the precursor. Or, the original fatty acid may undergo beta oxidation, the resultant degraded monocarboxylic acid being then oxidized by omega oxidation with the formation of a dicarboxylic acid containing two carbons less than the original. These reactions continue until a four carbon dicarboxylic (succinic) acid is produced. A direct pathway for the conversion of fatty acids to carbohydrate is thus provided since all four-carbon dicarboxylic acid are carbohydrate precursors.

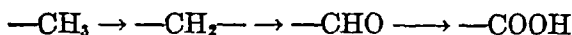
The evidence upon which this hypothesis is based is devious and Verkade's summarizing lecture should be consulted for the finer details

1 In man, following ingestion of monocarboxylic acids, di-acids are found in the urine But, this property is confined to the lower ones in the series viz

MONOCARBOXYLIC ACID-CARBONS	DIACIDOGENIC PROPERTIES
8	Weakly
9	Weakly
10	Strongly
11	Strongly
12	Slightly or none
13	None

In dogs, however, the diacidogenic properties of  $C_7$  to  $C_{11}$  acids was difficult or impossible to demonstrate In explanation of this difference, the difficulty of producing ketonuria in dogs is cited, and the lack of diacidogenesis is "possibly related and to be expected with the carnivora "

2 Methyl oxidation is well described in the literature and di-carboxylic acid formation is simply one example of it The probable mechanism is over some route such as



3 Although the lower dicarboxylic acids ( $C_6$ - $C_{10}$ ) when administered or injected are largely excreted unchanged, the higher ones are apparently catabolized since they fail to appear in the urine e g ,

DICARBOXYLIC ACID FED (DOG)	% EXCRETED UNCHANGED
$C_6$	58
$C_8$	45
$C_{10}$	33
$C_{11}$	17
$C_{12}$	$\pm 0$
$C_{14}$	0

Apparently the combustibility of these acids by the dog rapidly increases on ascent of the homologous series The relative non-combustibility of the lower dicarboxylic acids, which are presumably intermediaries in the catabolism of the higher ones, is explained with difficulty

4 Following ingestion of the dicarboxylic acids ( $C_6$  to  $C_{14}$ ), the homologous acids with 2, 4, or 6 less carbons can be recovered from the urine indicating that the original acids are degraded by beta oxidation However, direct evidence for omega oxidation of the higher fatty acids is still missing On this point Verkade stated "I have been unfortunately as yet to be satisfied with having pointed out the general character of the methyl-group oxidation "

5 Omega oxidation is related to carbohydrate oxidation In a fasting subject (man) ingesting triundecylin, the observed excretion of dicarboxylic acids

is significantly increased by the subsequent ingestion of glucose. This becomes a central part of Verkade's theory of ketosis. To explain why ingested dicarboxylic acids do not yield ketone bodies during fasting, he looked upon the diminution of diaciduria as a "pathological fact," for when carbohydrate is not available "the classical way of degradation (unilateral beta oxidation) is promoted at the expense of the way of degradation by omega oxidation with subsequent bilateral oxidation."

6 The demonstration of omega oxidation permits it to be stated "nearly with complete certainty" that the food fats are the "parent substances of the  $C_4$  system. Indeed, on degradation of these acids in the manner mentioned, succinic acid appears as an intermediate product."

7 This formation of succinic acid from fat immediately opens a pathway to carbohydrate and Verkade states that there can "no longer be any doubt of the possibility of this process."

It is difficult to evaluate this evidence as presented by Verkade. The fact that certain hypothetical intermediaries are catabolized does not constitute proof that fat metabolism normally goes through these channels, for many substances not remotely related to mammalian metabolism are readily catabolized. Certain weaknesses of the theory are evident. 1 Spontaneous diaciduria (except for traces of oxalic acid) does not occur. 2 Ketosis is difficult to explain since, apparently, ketone bodies do not form from dicarboxylic acids. For example, Edson (57), Mazza (121) and also Califano (29) found no ketone body formation by liver slices in the presence of representative dicarboxylic acids. A subsidiary hypothesis must be postulated that ketosis is a pathological process subsequent to the failure of normal omega oxidation which does not produce ketones. The recent evidence of Shipley (157) that ketone formation in considerable amounts occurs in livers from normal *fed* rats is strongly opposed to this explanation. 3 Finally, omega oxidation requires *ipso facto* the advocacy of the occurrence of carbohydrate formation from fats. The difficulties of maintaining this position are discussed elsewhere (fats to carbohydrates).

Flaschenträger and his co-workers (64, 65), have reported at length on the subject, and while agreeing with Verkade in some details, differ radically in interpretation of the evidence with respect to the quantitative significance of omega oxidation. In general, Flaschenträger's conclusions are that omega oxidation takes place to a very small degree in the case of the higher fatty acids (less than 1 per cent of total fat oxidation) and that its physiological significance is minimal. Bernhard (12) takes even a stronger view. Incorporating deuterium into dicarboxylic acids, he finds that succinic acid is readily catabolized when fed to dogs or rats, but that adipic, suberic, sebacic, and higher dicarboxylic acids so labeled with deuterium are not metabolized to any appreciable extent. He concluded that omega oxidation is not a step in the normal catabolism of fatty acids.

**CATABOLISM OF ODD FATTY ACIDS** The well-established fact that mammalian tissues contain no detectable amounts of odd numbered fatty acids would appear at first sight to deprive them of any physiological interest. On the contrary, all

CO<sub>2</sub> production upon addition of aceto-acetate Kühnau (102) reported that added aceto-acetic acid in the presence of liver is decreased with the formation of a series of reaction products, viz aldol, acetoacetate, acetaldehyde, succinic, fumaric, malic acids and traces of acetic acid

From the weight of the evidence, it must be concluded that the liver while forming ketone bodies does not utilize them to any significant extent in its own metabolic activities

*Muscles* Abundant evidence has accumulated that ketone bodies are freely used by the normal muscle tissue Experiments with perfused surviving limbs of experimental animals were done by Griesbach (76), Snapper and Grunbaum (162), and Toennissen and Brinkman (179) Eviscerated preparations have been used (Friedmann, 67, Chaikoff and Soskin, 33, Dye and Chidsey, 55), and utilization calculated from blood and tissue ketone body levels following injection of acetoacetate or  $\beta$ -hydroxybutyrate The evidence uniformly points to a complete oxidation of the substrate Careful measurements by Blaxter-Moeller (16), who determined both the ketone body utilization and oxygen consumption of perfused hind limbs of the cat in the resting state and when working (electrical stimulation), gave as the mean of 11 determinations a ratio of 3.6 moles of oxygen to one mole of acetoacetate consumed This is remarkably close to the theoretical value of 3.5 and proves that the substrate is completely oxidized

The arterio-venous ketone difference of certain organs has also been used to show ketone body utilization Goldfarb and Himwich (73) in such studies reported that striated muscle and heart utilized ketones, but that brain did not Barnes and Drury (49) also demonstrated the utilization of ketones by peripheral tissues by this method

The calculation of peripheral utilization from the difference of hepatic ketone production and urinary excretion has been successfully employed, the assumption being made that the excess of the former over the latter represents utilization In normal and diabetic cats Blaxter-Moeller (16) showed that the perfused liver produced ketone bodies greatly in excess of the preliminarily determined ketonuria Likewise Stadie et al (168) using surviving liver slices to determine hepatic ketone production, obtained the same results Crandall and his co-workers (40) inserted cannulae into the hepatic veins of dogs and calculated hepatic ketone production from the blood ketone values so obtained using estimated hepatic blood flows

Peripheral utilization has also been determined by following blood ketone levels after the injection of acetoacetate or  $\beta$ -hydroxybutyric into nephrectomized animals (Nelson, Grayman and Mirsky, 137) When followed by an analysis of the entire carcass this method becomes quite quantitative Numerous investigators have studied peripheral ketone utilization by the determination of urinary ketone body excretion alone or associated with studies of blood levels This method has a limited applicability because there is no quantitative relationship between hepatic ketone body production and urinary ketone excretion or blood levels For example, Stadie et al (167) found in diabetic cats that there

may be an enormous hepatic ketone body production with no or minimal ketone excretion. Harrison and Long (78) employed a unique method for determination of ketone utilization by the muscle. From blood levels they calculated ketone body concentration in cellular water. Even when ketones were high following phlorhizin or anterior pituitary injection, they find no significant amount of ketone in muscle cell water except at high concentrations, thus indicating that muscle metabolism of ketones was efficiently active to maintain them at practically zero level within the cell.

**Heart-lung** Using the isolated heart-lung preparation, Barnes, MacMoe and Visscher (10) first conclusively showed that this preparation utilized ketone bodies. Both the lung and the heart are active in this utilization; a considerable fraction of their total metabolism being thus accounted for. Recently, Merlini (124) demonstrated the utilization of acetoacetate and hydroxybutyrate by the isolated rabbit heart.

**Kidney** Perfusion studies with animals and human kidneys have shown ketone body utilization (Snapper and Grünbaum, 161). Using slices from dog and guinea pig kidneys, Quastel and Wheatley (142) showed that added acetate was oxidized away; they localized this action to the cortex. They made the interesting finding that the addition of lactate and glucose markedly increased the rate of ketone body utilization. More recently Shipley (157), using slices of kidney from fasting rats, showed that the rate of decrease of ketone bodies far exceeded that of any organ studies. He further showed the kidney gram for gram exceeded the liver in its ability to synthesize carbohydrate from non-carbohydrate sources. The relation, if any, of this to fat metabolism is undetermined.

**Brain** The general statement that brain metabolism is solely carbohydrate in character cannot be regarded as proven. Respiratory quotient determined on both the intact organ, and with brain slices and emulsions, consistently gives values of about 0.95 indicating that metabolites other than carbohydrate are being oxidized. Mulder and Crandall (134) were unable to demonstrate ketone body utilization of brain *in situ* following production of ketosis by fasting. However, they employed the method of arterio-venous differences which is sufficiently sensitive for this purpose. Shipley (157), by adding acetoacetate to brain preparations *in vitro*, demonstrated its total oxidation at a rate similar to that observed in muscle. This finding raises interesting questions as to the role of metabolism of the brain in the fasting or diabetic state.

**Adrenals** Boda (20) reported the interesting observation that 30 per cent of the ketone content of the adrenal vein blood is removed in its passage through the adrenals of the dog. He calculated that approximately 83 mgm/kgm weight/minute are utilized by the adrenals—a large proportion of the ketone utilization of the animal.

**Phlorhizin poisoning** Abundant ketone body utilization during acidosis induced by phlorhizin has been shown by Barnes, Drury, Greeley and Wick using the method of arterio-venous differences. Harrison and Long (78) showed the same thing from their calculation of ketones in muscle cell water.



*Exercise* The interesting problem as to whether the increased demand on working muscle can be furnished by ketone body utilization was answered equivocally by Blixenkrone-Moeller (16). In his perfusion studies on isolated surviving hind limbs of cats, he brought about a 10-fold increase in ketone body utilization by causing the muscle to exercise (electrical stimulation). When in a state of fasting ketosis were made to work heavily, Drury, Wick and Macdonald (54) found a sharp fall in blood ketone levels followed 2 or 3 hours later by a rise. They concluded that exercise first causes an increased ketone utilization by muscle, after a lag period there is an increased production by the liver to meet the extra demand. The work of Mirsky and Broh-Kahn (128), showing increased metabolism due to dinitrophenol is accompanied by increased peripheral ketone body utilization is corroborative evidence on this point. Neuhauser and Ross (136) also found evidence in exercising man that ketone body utilization is increased.

*Diabetes* For a period of forty years the convergence of two theories concerning fat metabolism dominated the question of the utilization of the ketone bodies by the diabetic organism and popularized the application of the aphorism "the solution of the problem". These theories dovetailed neatly one into the other. The theory of successive beta oxidation explained how fatty acids were degraded two carbon atoms at a time leaving a ketone residue, the two carbon unit split off (acetic acid by presumption) sufficed in the main for the metabolic need of the complete diabetic. The theory of obligatory coupling required that ketone body oxidation be coupled with that of carbohydrate. The combined theories explained the ebb and flow of the ketosis of the diabetic. Imagination likened the process to the "flame of the carbohydrate" which completely consumes fat, or the fat engine which "smokes" with ketones when diabetes mal-adjusts the carburetor. A third theory, different in nature, supposed that, owing to insulin lack, fatty acids are abnormally converted at a prodigious rate into glucose as well as ketone bodies both of which are excreted because their rate of production by the liver is greatly in excess of the ability of the peripheral tissues to utilize them. Chalkley and Soskin (33) first conclusively showed that the diabetic dog burned ketone bodies as readily as did the normal animal. They injected acetoacetate into a eviscerated, depancreatized animal and studied the rate of disappearance. Their experiments were repeated and confirmed (Dye and Chidsey, 55, Friedmann, 1931, Mirsky and Broh-Kahn, 127). Blixenkrone-Moeller (16) showed in a convincing manner in perfusion experiments on the hind limbs of diabetic cats an actual utilization of ketone bodies which was increased 3 to 5 fold during exercise. The oxygen consumption measured during rest and work indicated that all the ketone bodies were completely oxidized. He also showed, by a comparison of the rate of ketone body formation from perfused livers of diabetic cats and of preliminarily determined urinary ketone body excretion, that there was a great excess of ketone formed which was undoubtedly burned in the peripheral tissues. Stadie (165, 166) reviewed the subject and showed in the following ways that the diabetic animal utilized ketone bodies freely despite the complete absence of insulin, and with no relation to the oxidation of carbohydrate. 1, the ketone

production by liver slices from diabetic cats was greatly in excess of the ketone excretion in the urine determined in a preliminary period the excess represented ketone utilization, 2, muscle tissue from diabetic cats equilibrated with acetoacetate oxidized it away at a rate sufficient to take care of a considerable fraction of the total energy requirements, 3, ketone bodies produced by liver slices from diabetic cats were oxidized away when the slices were simultaneously equilibrated with muscle mince from the same animal, 4, hepatic ketone production measured by portal-hepatic ketone differences in diabetic cats was again greatly in excess of urinary ketone excretion, 5, by an analysis of the best metabolic data in the literature on human cases of diabetes treated before the days of insulin when ketosis was a common occurrence, Stadie concluded that the diabetic could completely catabolize about 2.5 grams of fat per kgm. body weight per day without ketonuria. Since a considerable part of this total fat catabolism went by way of preliminary partial oxidation to ketone bodies in the liver, it was clear that the human diabetic could oxidize ketone bodies freely, independently of insulin or carbohydrate oxidation. A summary of these data is given in the accompanying table 5.

**THE MODE OF OXIDATION OF FAT IN MUSCLES** The problem of what metabolites are completely oxidized to supply energy for the muscles in rest and exercise has been of major interest to physiologists for many years. The older idea that carbohydrate is the sole muscle fuel had to be abandoned in view of the conclusive evidence that muscles oxidize the ketone bodies freely. The question as to whether or not fatty acids per se could be oxidized directly has been a subject of controversy, and Gemmill (71) in his recent review of the fuel for muscular exercise concluded that "There is no experimental evidence at the present time for the direct utilization of fat by mammalian muscle." The present reviewer cannot agree with this position. In an evaluation of the evidence (165) he proposed that the total fat catabolism could be represented by

a. *Indirect oxidation*, i.e., a preliminary partial oxidation of fatty acids in the liver to ketone bodies followed by complete oxidation of the ketones in the periphery (chiefly muscle), and (b.) *direct oxidation*, initiated and completed in the periphery. He found (165) with liver slices from depancreatized cats, ketone body formation equivalent to 0.09 gram of fat per kgm. of body weight per hour whereas the total metabolism of the diabetic cat was of the order of 0.32 gm / kgm./hr, i.e., only about 30 per cent of the total metabolism could be accounted for by indirect fat utilization. The balance must have been by direct oxidation, unless it was assumed that the diabetic cat could utilize glucose. These values for ketone utilization were in substantial agreement with those of Blixenkrone-Moeller (14) using perfused livers from diabetic cats. Crandall, Ivy and Ebni (40) came to a similar conclusion when they estimated the hepatic ketone production in angiotomized dogs. After five days of fasting when all of the available carbohydrate stores were used up, they estimated that only about 50 per cent of the total energy requirements of the animals could have been met by the ketone bodies formed in the liver. The balance must have been by direct oxidation of fats in the muscle.

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Another difficulty when the possibility of fat oxidation by the muscles is excluded has been pointed out by Stadie (165) If the entire muscular metabolism during exercise in the fasting state when carbohydrate stores are depleted, is dependent upon indirect fat oxidation, the liver will have to increase its oxygen

TABLE 5

*Calculation from data in the literature of ketone body utilization by the peripheral tissues of the normal and diabetic animal*

	MEAN KETONE UTILIZATION PER KG. PER DAY
	<i>gram</i>
Chaikoff and Soskin (33)	
By measurement of rate of disappearance of injected ketone bodies from blood of	
Diabetic eviscerated dogs	2.9 ± 0.1
Normal eviscerated dogs	3.4 ± 0.6
Friedmann (67)	
By measurement of ketone body excretion when injected with very large amounts of acetoacetate	12 (maximum)*
Blivenkrone-Moeller (16)	
By comparison of ketone formation of perfused diabetic cat livers with prior ketone excretion	3.9 ± 0.3
By measurement of rate of disappearance of ketone bodies from blood perfused through	
Resting hind limbs of normal and diabetic cats	2.7 ± 0.4
Working hind limbs of normal and diabetic cats	13.0 ± 0.7*
Dye and Chidsey (55)	
Injection of acetoacetate at high rate into nephrectomized-depancreated dogs (maximum)	18 *
Stadie, Zapp and Lukens (167)	
1 By comparison of ketone formation by diabetic cat liver slices with prior urinary ketone body excretion	2.7 ± 0.4
2 By measurement of ketone body utilization by diabetic cat muscle mince in presence of added acetoacetate	2.2 ± 0.6
3 By measurement of ketone body utilization of diabetic cat muscle mince simultaneously equilibrated with diabetic cat liver slices	5.0 ± 1.0
4 By measurement of the portal-hepatic ketone difference in diabetic cats	3.0 ± 1
Mean basal ketone utilization	3.0 ± 0.3
Equivalent grams of fat	2.3

\* Excluded from basal mean

uptake very greatly to form the necessary ketones In a hypothetical case the hepatic oxygen uptake during moderate exercise was calculated to be approximately 1000 micromoles per gram per hour, ten times that determined for the basal state (Bancroft and Shore, 5) It is difficult to suppose that the oxygen uptake in the liver ever attains such high levels

Crandall and his co-workers (37, 40), by studies of hepatic ketone production in the angiotomized liver and by blood ketone studies, describes a marked lag in hepatic ketone production upon fasting in dogs and also man. Only after 2 to 3 days does hepatic ketone production reach maximum levels, and even then it is only sufficient to account for about half of the total metabolism. In the face of this lag and the loss of available carbohydrate stores, it is hard to explain how the metabolic requirement can be met without supposing direct fat oxidation in the muscles.

Bearing on the question are the experiments of Barker (6) who found no increase of ketonuria or protein metabolism following exercise in diabetic dogs. To supply these extra calories one would have to suppose an instantaneous and large increased production of ketones to extraordinary heights by the liver, or a marked increased carbohydrate metabolism, or direct fat oxidation. The evidence is very much against the first two alternatives.

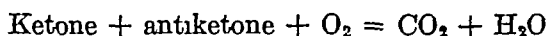
Nor is more direct evidence lacking. Barnes, Drury, Greeley and Wick (8) measured the peripheral ketone utilization and oxygen consumption by arterio-venous differences in phlorhizinized dogs. They could only account for approximately half of the metabolism by ketone utilization. In studies on heart-lung preparations (10) accompanied by  $\beta$ -hydroxybutyrate injections they calculated that the heart could oxidize fats directly to the extent of  $\frac{1}{3}$  of its metabolic requirements.

Studies of the respiratory quotient of muscles in the fasting state either in vitro (138) or in the intact state (81) give values close to the theoretical value of 0.7 for oxidation of fat. Cruckshank (43, 44), who spent years perfecting his ability to handle the heart-lung preparation for metabolic studies, found repeatedly in diabetic hearts R.Q. values of 0.7 which promptly rose to 1 following the injection of insulin. Those who are skeptical of the significance of R.Q. values in isolated tissues must explain, if they exclude direct fat oxidation, why diaphragms from fasting rats when equilibrated without glucose almost invariably show R.Q. values close to 0.7. Drury and McMaster (53) contributed valuable evidence by determining the R.Q. of rabbits deprived of their livers. Following fat feeding the R.Q. was not significantly changed from the preliminary period before hepatectomy. Their average R.Q. value in the preliminary period was  $0.73 \pm 0.01$ , and remained unchanged even as late as 24 hours after the operation. Apparently, the organism deprived of liver is still capable of burning fat independent of any possible formation of carbohydrates or ketones by prior action of the liver. In other words, the low R.Q. is a true fat combustion R.Q. and not the algebraic sum of processes going on in the liver and muscles simultaneously. Gemmill's own work (70) in which he determined the fat and carbohydrate content of phlorhizinized rat muscle in the resting and exercising state cannot be used as proof that muscle is unable to oxidize fat. He found a decrease of carbohydrate but none of fat. However, his data do not include analyses of blood which must be considered as a possible source of fat. Furthermore, phlorhizinized muscle is still capable of oxidizing glucose, hence his preparations might not have been suitable ones for the demonstration of direct fat combustion. But even if the sum of this evidence was insufficient, the direct evi-

Cruickshank and Kosterlitz (42) remains. In many experiments on dogs' hearts, they measured the total initial and final phospholipids, fatty acids, and cholesterol. When there was low cardiac glycogen to favor fat oxidation, they were able to demonstrate beyond doubt that the heart can and does utilize stored fatty acids per se for its metabolic needs. This confirms the older observations of Visscher (181) who, on the basis of oxygen and carbohydrate balance studies, had made the same conclusion.

**THE INTEGRATION OF CARBOHYDRATE AND FAT METABOLISM** *Ketolysis, anti-ketogenesis, and the ketogenic-antiketogenic ratio*. It has long been known that there is a close integration of carbohydrate and fat metabolism, the mechanism of which, despite a multitude of experimental papers and hypotheses, still remains obscure. In fasting or in diabetes mellitus carbohydrate oxidation is diminished or absent, hence fat becomes the major metabolic ingredient. Upon restoration of carbohydrate oxidation, either by the ingestion of it, in fasting, or the injection of insulin, in diabetes, fat metabolism recedes into the background, and the metabolic mixture becomes predominantly of a carbohydrate character. The explanation of this phenomenon has stimulated the advocacy of contending hypotheses only one of which can safely be said to be eliminated from consideration.

*The obligatory coupling of carbohydrate-fat oxidation ketogenic-antiketogenic ratio*. This theory, which goes back as far as Gaelmuyden and was in reality a complement of the successive beta oxidation hypothesis, explained the recession of ketonuria following resumption of carbohydrate oxidation in diabetes mellitus and fasting. It supposed that acetic acid or other two carbon compound split off by hepatic beta oxidation of fatty acids, could be freely used in the periphery, but that the ketone residue could not unless its oxidation was directly coupled with the oxidation of carbohydrate or some "antiketogenic" intermediate, derived therefrom, viz



The assumptions were clearly stated by Shaffer (148) "Antiketogenesis in the human subject is based upon a ketolytic reaction in the body between acetoacetic acid, the first formed of the acetone bodies, and a derivative of glucose (or other antiketogenic substance), the compound being further oxidized, but that failing to react with ketolytic substances, acetoacetic acid is resistant to oxidation, accumulates, and is excreted. The fact that one finds at the threshold of ketosis an approximately constant ratio between the number of molecules of the precursors of acetoacetic acid and of glucose in the metabolic mixture, must mean that the further oxidation of acetoacetic acid constantly taking place under normal conditions is accomplished through a chemical reaction with a derivative of glucose."

The stoichiometrical ratio between the ketones and antiketones was termed the ketogenic-antiketogenic ratio and was usually stated to be 2:1. Innumerable attempts were made to measure this ratio experimentally in man and animals. Stadie (165) re-examined the best data on human diabetes available

in the literature. The 12 cases were all classical ones, reported before the advent of insulin when marked ketosis was, of necessity, a frequent accompaniment of the disease. The data include calorimetric measurements of the metabolic mixture of proteins, fats, and carbohydrates together with the total ketone excretion. Hence it was possible to calculate the total carbohydrate or so-called antiketones oxidized. In addition, from the total fat catabolized and the urinary ketone excretion, the total fat utilized or its equivalents in ketones could be calculated. His analysis showed that there was no significant relation between the fat utilized and the "antiketones" oxidized, i.e., the ketogenic-antiketogenic ratio had no reality. On the other hand, there were strong indications that these patients, many of whom were almost complete diabetics, were oxidizing ketones or fats without simultaneous oxidation of carbohydrates, i.e., obligatory coupling was unnecessary. Stadie stated a simple hypothesis of fat metabolism in diabetes mellitus which would conform to the observations in these clinical cases and with experiments with animals, viz up to a certain level all fat catabolized is completely oxidized, hence there is no ketonuria. Beyond this level (approximately 2.5 gm./kgm./day) all fat catabolized is not completely oxidized, hence part of the fat catabolized is excreted unburned in the form of ketone bodies. The action of carbohydrate is simply to spare fat oxidation, there is no molecular reaction, hence there is no molecular ratio between the two.

The work of others who have measured directly in experimental animals the simultaneous utilization of ketone bodies and carbohydrates when intravenously injected, has shown that the rate of utilization of one is not affected by that of the other (130, 67). In particular, Dye and Chidsey (55) determined total ketone body and carbohydrate utilization in nephrectomized-normal and nephrectomized-depancreatized dogs following the intra venous injection of acetoacetate and glucose in varying amounts. From their data they found the ratio between these two utilizations to vary from 0.7 to 19.6. Furthermore, the rate of utilization of ketone bodies was independent of the blood sugar level, carbohydrate utilization, evisceration, or pancreatectomy, being solely a function of the concentration of blood ketones.

Waters, Fletcher and Mirsky (184) came to equally decisive conclusions. They studied carbohydrate and ketone utilization in the dog heart-lung preparation and found no positive correlation between the two. Their "ketogenic-antiketogenic ratios" varied from 0.2 to  $\infty$ .

The ketogenic antiketogenic ratio has proved to be an ignis fatuus. It is the belief of the reviewer that there is no evidence for the obligatory coupling of ketone-carbohydrate oxidation and that a strictly stoichiometrical, small whole number ratio between ketogenic and antiketogenic substances is non-existent.

**Ketolysis.** On the other hand, the ideas behind the theory of ketolysis cannot be dismissed lightly, particularly in view of recent work indicating a possible integration of fat and carbohydrate metabolism involving citric acid and the four carbon dicarboxylic acids (v. infra. Wieland and Rosenthal, 191, and Breusch, 22). A careful distinction should be made between "ketolysis" and "obligatory



coupling" The latter implies a strict simple molecular ratio between two substances when reacting, without the reaction no oxidation whatever of ketones occurs The former is not limited to this, but might include the case in which fat metabolism already occurring independently might be *accelerated* by interaction with carbohydrate intermediaries It is conceivable that by mechanisms still undefined carbohydrate would bring about further oxidation of ketone bodies or even fatty acids in unlimited molecular ratio In other words carbohydrate might act "catalytically" in promoting the oxidation of fat or its intermediaries

For example, Shaffer (148) demonstrated in model experiments *in vitro* that under certain circumstances glucose catalysed the oxidation of acetoacetate Shapiro (150) found in feeding experiments with rats that only glucose formers were effective in diminishing the ketonuria following acetoacetate injection

The same results have been obtained with amino acids (Butts, Dunn and Hallman, 28, Butts, Blunden and Dunn, 25) Deuel (48, 49) has engaged in a sharp polemic with Mirsky (129, 130) on this subject and has stuck staunchly to the position that the phenomenon of ketolysis is real He criticized the opposing experiments of Mirsky on technical grounds and cites his own work in support He found in rats a greater rate of disappearance of injected 1- $\beta$ -hydroxybutyrate when glucose was simultaneously given Deuel also studied the endogenous ketonuria in rats on prior high fat diet, and following administration of butyric acid Glucose—but not an isodynamic amount of alcohol—significantly diminished ketonuria in both cases More significant is his calculation that in the endogenous ketonuria of fasting, the administration of a small amount of glucose iso-dynamically equivalent to approximately 2 per cent of the fat catabolism resulted in the excretion of an average of 3.2 mgm per 100 gm /d compared to 43.2 gm /100 gm /d in the controls Bobbitt and Deuel's (19) experiments with liver slices in the presence of butyrate without or with added glycogen must be viewed with more caution particularly since they are somewhat opposed to those of Edson (58) They concluded from their data that in the presence of glycogen more butyrate disappeared and less ketone bodies were formed indicating a possible "catalytic removal of ketone bodies by carbohydrate, either by oxidation, or by formation of compounds which do not react with Deniges reagent" But their calculated butyrate decrease was the small difference between two large numbers, and in their balance they did not consider the possibility that butyrate might be converted to glucose (Blixenkrone-Moeller, 15, Buchanan, Hastings and Nesbitt, 23)

In view of this evidence and the recent developments in intermediary fat metabolism in the periphery, the reviewer feels that it would not be wise to exclude a possible effect of carbohydrate catabolism upon that of fat by some mechanism which could be described by the term "ketolysis" in some more far-reaching sense than the usual one

*Substrate competition* In discussion of the integration of fat and carbohydrate metabolism, substrate competition plays a rôle Edson (58) discussed it at some length The supposition is that tissues oxidize one substrate in preference to

another, there is no significant change in total oxygen uptake since this is limited by relatively non-specific mechanisms. For example, the deamination of 1 amino acids by surviving kidney slices is diminished in the presence of a readily oxidizable substrate such as lactate or pyruvate. Quastel and Wheatley (141) found that propionic acid, but not glucose or lactic acid, inhibited the formation of acetoacetic acid from butyric acid by liver slices. Edson (58) found, using liver slices, that spontaneous ketone formation can be significantly altered by the addition of certain substrates without, as expected from the theory, there being any great change in total oxygen uptake. Glycerol, sorbitol, glyceraldehyde were strongly antiketogenic, whereas glucose, fructose, mannose, galactose were moderately so, and glycogen not at all. This antiketogenic effect is a simple "sparing" of fat and does not involve any direct chemical interactions.

Stadie et al (167) studied the effect of fructose in diminishing the ketone formation of liver slices from diabetic cats. Their results are summarized in table 6.

TABLE 6

*Average decrease (per cent) from control of ketone formation by liver slices from diabetic cats  
Micromole per gram per 2 hours (Stadie, Zapp and Lukens, 167)*

INSULIN	FRUCTOSE	FRUCTOSE + INSULIN	FRUCTOSE + FUMARATE	FRUCTOSE + FUMARATE + INSULIN	FUMARATE	FUMARATE + INSULIN
+9	-12	-23	-20	-42	-28	-26

The decrease effected by fructose was always enhanced by the presence of insulin, but the greatest effect was found with fructose, fumarate and insulin. They also found that the effect of fructose and insulin was enhanced if the slices were equilibrated for a longer period of time (4 hrs). These results, while indicating a possible substrate competition by fructose enhanced by insulin, also indicate involvement of the four carbon di-carboxylic acids in fat catabolism as originally suggested by Szent-Gyorgyi (176).

*Hepatic glycogen and ketone formation* The inverse relation between the glycogen content of the liver and its rate of ketone formation has long been known. Blixenkrone-Moeller (14) in his discussion of this topic pointed out the following relations: 1, in general hepatic ketone production tends to be higher in livers with the greatest fat content, 2, more significant is the glycogen content. In normal animals fasting brings this level down to about 1 per cent and there is moderate ketone formation, in depancreatized animals having hepatic glycogen levels less than 1 per cent, ketone production is highest irrespective of the level of liver fat, 3, when in normal animals liver glycogen is brought below 1 per cent by the using of adrenalin or phlorhizin, ketone production is much higher but even then does not consistently reach the levels attained by diabetic livers. Blixenkrone-Moeller eliminated as a possible explanation for this inverse correlation of glycogen and ketone formation "sparing" of fatty acid oxidation by glycogen. For upon correction of oxygen uptake for ketone formation, he finds

no difference in the R Q of glycogen-rich or glycogen-poor livers Stadie et al (167), found essentially the same thing Corrected in the same way the R Q of liver slices from diabetic cats was not significantly different from that of normal cats, ( $0.81 \pm 0.05$ ) When hepatic ketone formation was markedly reduced by adequate treatment with insulin in a preliminary period, the non-ketone R Q of liver slices was lowered rather than raised

On the other hand, Mirsky (129) and his co-workers studied ketonuria in diabetic dogs before and after massive doses of glucose intravenously and came to opposite conclusions They stated that their "data are in accord with our working hypothesis, that the production of ketosis by the liver itself is always due to a decrease in the amount of glycogen available for utilization by the liver itself and that glycogen formation is an obligatory step in the oxidation of carbohydrate and the prevention of ketosis The production of ketone bodies is attributed to a compensatory acceleration in the metabolism of fatty acids and protein in the liver in consequence of a decrease in the amount of carbohydrate available for oxidation by the liver itself" The reviewer knows of no evidence requiring the formation of glycogen from glucose prior to oxidation In fact, all schema of glycolysis place hexose monophosphate as a common intermediate step between glucose, glycogen and the products of glycolysis Hence, if this type of substrate competition explains the suppression of hepatic ketosis, glucose itself, which is abundant in the blood in diabetes, should be just as efficient an antiketogenic agent, which it is not Other facts must also be considered 1 The shorter fatty acids when administered give rise to abundant ketone formation even in the presence of abundant readily available glycogen in the liver (116), 2, there is a lag in maximum ketone production in certain species on fasting (v supra), i e, maximal ketone production is reached long after hepatic glycogen has reached its lowest levels, 3, ketone production continues after liver glycogen has been raised by feeding (Wick and MacKay, 189), 4, hepatic ketone production in Houssay cats is significantly lower than that of normals despite the almost complete absence (0.03 per cent) of glycogen in the liver (Stadie et al, 167)

In a purely speculative paper Witzeman (193) reviewed the evidence and formulated a hypothesis bearing on the problem of reciprocal integration of carbohydrate and fat catabolism He summarizes the pertinent evidence in 20 categories Unfortunately much of recent evidence is ignored, and in instances questions which are still in doubt are categorically asserted to be proven In other instances, evidence cited has been controverted by more recent work Nevertheless his discussion of fat catabolism is interesting, but the finer details of it cannot be considered here According to Witzeman, fat metabolism embraces the following events 1, transportation of fats by conversion to hydrophylic phospholipids and cholic acid-cholesterol complexes, 2, combination of these hydrophylic compounds with oxidizing enzyme systems concerned with fat catabolism, 3, oxidative mechanisms bringing about the two typical end results of fatty acid catabolism a, complete combustion, b, production of ketone, 4, "recapture" of soluble fatty acid intermediaries (acetic acid, aceto-acetic

acid, etc.) and oxidation by enzyme systems primarily involved in carbohydrate metabolism. Such a system, presumably by mass action effects, might integrate carbohydrate and fat metabolism. Although speculative and dogmatic, Witze-man's paper repays study. At least one can echo his hope "that the general catalytic channels have been worked out in amazing detail in the carbohydrate physiology we can scarcely expect anything less satisfactory for fat, when the story is told."

The integration of carbohydrate-fat metabolism must await further experimentation. Substrate competition, endocrine influences, cyclical involvement of the four carbon dicarboxylic acids, phosphorylations, are some of the factors which must be involved in the complete theory.

**FAT TO CARBOHYDRATE** This question continues to be a cause celebre among biochemists, but certain new lines of thought have arisen which may reconcile old conflicts. The liver has hitherto believed to be the sole source of blood sugar, but the kidney has now advanced to the front rank as a glucose former. Information about its importance in this rôle is still meagre. The evidence that the diabetic or phlorhizinized liver does not convert fatty acids to carbohydrate is very strong, but no such dogmatism can be maintained with respect to the normal liver. Involvement of the four carbon dicarboxylic acids in the peripheral oxidation of fatty acids is being actively discussed. Perhaps this foreshadows a transformation of fatty acids to carbohydrate intermediaries which keeps pace *pari passu* with oxidation, so that outpouring of glucose into the blood from peripheral sites never occurs. The use of isotopic carbon permits the establishment of definitive pathways of metabolism, categorical answers to multitudinous questions concerning fat metabolism may be found by this means.

There are several reviews devoted solely to the subject (131, 165). The evidence pro and con can be grouped under the following heads:

1. Type of fuel for muscular work
2. Total non protein R.Q. in rest and exercise
3. D/N ratios. Normal, diabetic, adrenalized, or phlorhizinized animals, organs, or isolated tissues
4. Hepatic oxygen ketone ratio
5. Intermediate pathway of fatty acid derivatives

**Fuel for muscular work** Many early workers assumed that muscle utilized carbohydrates only, hence a conversion of fat to glucose by the liver was obligatory. In exercising subjects the loss of muscular efficiency in subjects fasting or on fat diets was assumed to be due to the loss attending this conversion. The possibility which we now know to be real that the conversion was to ketones never considered (Krogh and Lindhard, 1911). The both direct  
indirect oxidation of fat by muscle has been reviewed.  
can no longer be regarded as the sole fuel of muscle

**Total non-protein respiratory quotient** The total oxidative processes throughout the body. Assume hject  
pleted carbohydrate stores to be converting fat to the

non-protein R Q will be that of pure fat oxidation (0.7) unless *a*, there is a loss of glucose or ketone bodies in the urine, as in diabetes or phlorhizin poisoning or, *b*, there is storage of carbohydrate as glycogen in muscles of liver, or of  $\text{CO}_2$  in the body buffers

An evaluation of the clinical and experimental data in diabetes fails to reveal well authenticated examples of non-protein R Q significantly below the theoretical 0.7 for complete fat combustion. Hawley (79) has reported a searching investigation on this subject using phlorhizinized dogs and determining the R Q and the D N ratio. She was unable to find any evidence indicating the conversion of fat to carbohydrate. In humans under exercise Stewart, Gaddie and Dunlop (173) came to the same conclusion from data on the R Q. Soskin (164), discussing the subject, gave weight to the small decreases of non-protein R Q below 0.7 reported in the literature as supporting the hypothesis of fat to carbohydrate. But granting that these small differences are real, they would indicate only a small conversion of fat to glucose, i.e., about 10 to 20 per cent of the total fat catabolized. In the average diabetic or phlorhizinized animal the formation and the excretion of 50-100 grams of glucose from fat would result in a marked decrease in the non-protein R Q well below 0.7 and outside of the range of possible experimental error.

Werthessen (188) trained rats to eat but once a day and measured the R Q at frequent intervals. In most instances the R Q was equal to or greater than 0.7 but there were occasional sharp brief changes to 1.7 or 0.3. The author concluded that these changes indicate a breakdown of total metabolism into steps. Without confirmation of these unusual and interesting findings with data on the  $\text{CO}_2$  and ketone storage or elimination, these findings cannot be evaluated in relation to the present problem.

*D N ratio* The situation here is the same as with the R Q. Any considerable fraction of total fatty acid catabolism resulting in glucose formation would make the D N ratio undisputably high. In ignorance of the exact proportion of protein convertible to carbohydrate under varying conditions, it is impossible to deny or assert from D N data available that a *small* fraction of the catabolized fat may be converted to glucose. The occurrence of well-authenticated D N ratio greater than 4 is rare. Soskin (164) found in depancreatized dogs D N ratios over 3.2 in only about 10 per cent of the observations. Chaikoff and Weber (31) found high D N ratios in dogs injected with adrenaline and concluded therefrom that carbohydrate was derived from fat, but Bollman, Mann and Wilhemj (21) showed clearly that the extra glucose came from the muscle glycogen, a result confirmed by Bachrach, Bradley and Ivy.

*Liver D N ratios* Here the evidence is equivocal. Burn and Marks (24), using perfused livers of normal fat fed and one diabetic dog, found extra amounts of glucose synthesis which they concluded came from fatty acids. However, Gregg (75) repeating these experiments failed to get consistent results and asserted that the distribution of residual glycogen was so variable that the use of perfused liver was a highly unsatisfactory method of studying the question. Blumkrone-Moeller (14) concluded that ketone formation from fatty acids

was the dominant reaction in diabetic cat livers. He nevertheless considered a small conversion to glucose possible. Heller (80) determined the hepato-portal differences of sugar and lactic acid in intact 3-day fasted normal dogs and compared them with the urinary nitrogen excretion. In comparison with the urinary nitrogen, he calculated an excess of sugar of 2.2 mgm./gm liver/hour, even on the assumption that all of the protein and lactic acid is converted into glucose. Conclusions involving an estimated hepatic blood flow, as does this one, have doubtful value, particularly in this case. For if Heller's estimation is too high by 20 to 30 per cent the calculated extra glucose vanishes. Jost (86) also perfused normal and diabetic livers with phospholipids (lecithin and cephalin). He observed a large increase of glucose and glycogen formation. These he attributed to conversion of phospholipid to carbohydrate. However, in depancreatized dogs, he was completely unable to demonstrate any extra formation of carbohydrate. This he explains away by stating "The transformation of ketones to carbohydrates needs the intervention of some intermediary metabolic product of carbohydrate oxidation" (Reviewer's translation).

*Hepatic oxygen-ketone ratios* In the diabetic liver, as contrasted with the normal, the metabolism is mainly that of fat oxidation. Hence the oxygen uptake, particularly if it can be corrected for other known oxidations, will give a maximum value for fatty acid oxidation. Since both ketone and glucose formation require oxygen, the  $O_2$  ketone ratio under these circumstances becomes a clear indicator of the course of the metabolism. Blixenkrone-Moeller (14) measured this ratio in perfused diabetic cat livers. He concluded that the main product of fatty acid oxidation was ketone bodies, but he did not exclude the possible formation of small amounts of glucose. The contention of Gemmell and Holmes (72) that the low R.Q. values observed with liver slices from fasting or fat fed rats is evidence for the formation of glucose from fatty acids cannot be accepted, since ketones formation was not measured.

Stadie, Zapp and Lukens (167) measured the hepatic oxidative metabolism of liver slices from diabetic cats. The sum of oxygen required for ketone and  $CO_2$  formation together with that required for oxidative deamination as determined from urea and ammonia formation, was not significantly different from the total observed oxygen uptake. In other words there was no oxygen available for glucose formation from fatty acids and the conclusion appeared inescapable that the diabetic liver oxidizes fatty acids to ketones only and no glucose whatever is formed in the process.

*Intermediary pathways of fatty acid derivatives* Attempts to demonstrate the conversion of acetoacetate to carbohydrate have universally failed. Deuel, Butts, Hallman and Cutler (40) were unable to demonstrate any significant hepatic glycogen deposition in rats fed acetoacetate, butyrate, caproate, caprylate, or oleic acid, although there was abundant formation following valeric, heptylic or nonylic acids. Further, they could find no evidence for transformation of acetic acid to glucose in phlorhizinized dogs (50). Stöhr (174) obtained the same results. Blixenkrone-Moeller (14) although convinced that the major portion of hepatic fat oxidation in diabetic cat livers resulted in ketone body

formation, still asserted the possibility of a small amount of glucose formation from fats. However, he could find no extra glucose formation when acetoacetate was perfused through livers. Stadie, Zapp and Lukens (168) re-examining the claim of Weil-Malherbe (185) that the kidney converted acetoacetate to glucose, could find no evidence for it. Furthermore, in balance studies the decrease of fatty acids in liver slices was fully accounted for by ketone body formation (169). Using liver slices which they showed could synthesize carbohydrate from butyric acid, Cross and Holmes (41) could show no carbohydrate formation from acetic acid. Haarman and Schroeder (77) claimed that liver slices convert di-hydroxy butyric acid to carbohydrate. Upon analysis, their data show an apparent synthesis under anaerobic as well as aerobic condition, an impossible result. Failure to remove acetone completely before lactic acid analysis may be the cause of this discrepancy.

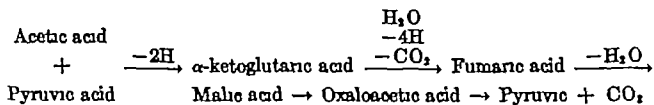
Butyrate appears definitely to be a precursor of glucose in the liver as has been shown by Blixenkrone-Moeller (15) in perfusion experiments, and more recently by Buchanan, Hastings and Nesbitt (23) using radio-active carbon in the carboxyl group. Hitherto, evidence for this reaction was lacking (Deuel, Butts, Hallman and Cutler, 46). Blixenkrone-Moeller (15) found no formation of glucose from valeric acid when it was perfused through normal and diabetic livers.

*Alternative pathways of hepatic fatty acid oxidation.* It is conceivable that in some circumstances, e.g., in diabetes, the liver oxidizes fatty acids to ketone bodies, while in others, e.g., in the normal state, the conversion is to carbohydrate. In other words, in the general reaction  $\text{Fatty acid} + \text{oxygen} = n \text{ ketone} + (1-n) \text{ glucose}$ ,  $n$  may vary from 0 to 1. This conception might reconcile conflicting data though the evidence for it is hardly more than vaguely suggestive. 1 The constant finding in diabetic or phlorhizinized animals of D/N ratios (ca 3/5) supporting the no carbohydrate hypothesis would be reconciled with the high (20 or more) ratios found in perfused normal livers (Burns and Marks, 24, Heller, 80). 2 Jost's (86, v supra) failure to find high D/N ratios in perfused diabetic in contrast to his findings with normal livers would be comprehensible. 3 Verkade's (186) inference that "ketone formation in the diabetic represents an abnormal departure from normal omega oxidation" would have more definite meaning. 4 The lag, often marked, of ketone formation in fasting animals, might mean to those who deny direct fat oxidation in the muscle that in the initial period of the fast the fuel for muscle energy is glucose from fat. Later the type of hepatic metabolism would change and ketones would be the main product of fatty acid oxidation. 5 The observation by Lehninger (108) that in fasting, acetylpyruvate is ketogenic, whereas in the hypoglycemia following insulin injection, it appears to be glucogenic indicates that one substance may follow two different metabolic pathways under two different metabolic conditions.

*Summary.* The reviewer concludes from evaluation of this evidence that fatty acids are not converted to glucose by the liver of the diabetic or phlorhizinized animal. The evidence that the reaction occurs in normal livers is meagre and indirect, nevertheless judgment must be suspended. No conclusive evidence

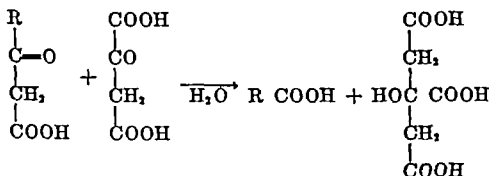
for the conversion in the kidney is at hand. The possibility that the muscles may form carbohydrate intermediaries during fat oxidation must be considered, but data in this field are still controversial. There is no evidence that such hypothetical carbohydrate intermediaries are precursors of blood glucose. If formed, they must be further oxidized *in situ*.

**INTERMEDIARY PATHWAYS OF FATTY ACID DERIVATIVES.** Edson and Leloir (59) studied the mechanism of ketone body metabolism in various tissues using slices. They found that pyruvate and fructose accelerated the anaerobic disappearance of acetoacetate in the liver but had no marked influence in other tissues except pigeon kidney. The reaction acetoacetate  $\rightarrow$   $\beta$ -hydroxybutyrate appears to be demonstrable in most tissues. With kidney tissues, acid accumulation suggested that acetoacetate breaks down through acetic acid (cf. Lehninger) and malonate inhibition suggested that the latter is oxidized by some such scheme as



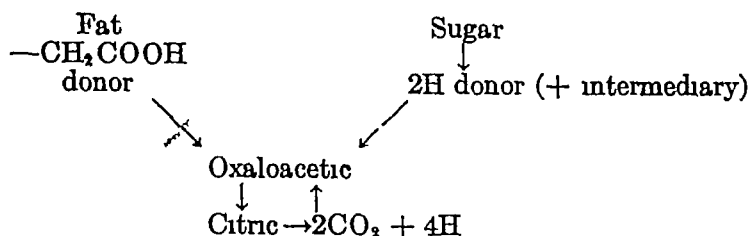
The possible pathway of acetoacetate oxidation via conversion to acetic acid has been investigated. Lehninger (107) using muscle minces and cell-free extracts of muscle and kidney reported "a formation of acetic acid from acetoacetic acid which is small and irregular but definite." He proposed three possible mechanisms the evidence being insufficient to choose among them: 1, hydrolysis, 2, phosphorylation into acetic acid via acetylphosphoric acid, and 3, dismutation of pyruvic acid which may be formed from acetoacetic. Host-Jorgensen (82) discussed the possible formation of acetic acid by dismutation of pyruvic acid which may be formed from acetoacetic acid. Barnes and Lerner (9) studied the metabolism of glycolic and glyoxylic acids in the rat. They concluded that the former is neither glycogenic nor ketogenic. Because the latter causes an increase of blood ketone in the fed rat, the authors suggested that it may be the two carbon unit formed by beta oxidation of fatty acids. Krael and Gibson (96) were unable to demonstrate oxidation of acetone by liver or muscle *in vitro*.

Two highly significant pieces of work have recently been reported which in the main concur. Breusch (22) in a short report without details discussed a new enzyme—citrogenase—which catalyzes the following reaction between beta keto derivatives of fatty acids and oxaloacetic acid

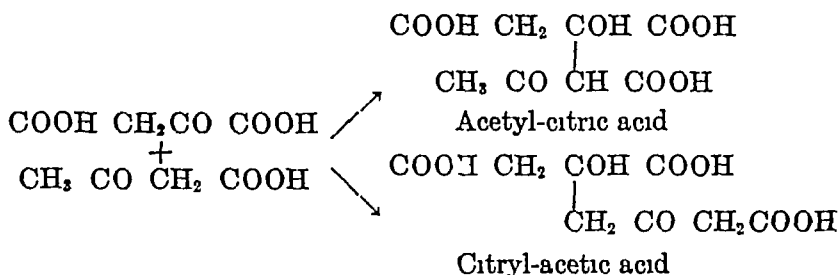




Beta keto acids (including dicarboxylic acids) are attacked with the formation of citric acid. The degraded residual acid is again oxidized, thus providing for complete oxidation of fatty acids by successive beta oxidations, the intermediates passing through a carbohydrate pathway. The enzyme was demonstrated in large amounts in muscle, kidney and brain, but little in liver, and none in spleen, pancreas or lung. Breusch proposed that oxaloacetic acid is the point common to fat and carbohydrate oxidation as follows:



Wieland and Rosenthal (191), following up the implications of the work of Sunderhof and Thomas (163) with yeast, reported experiments indicating the condensation of acetoacetate and oxaloacetic acid leading to the formation of a procitric acid according to the alternative scheme:



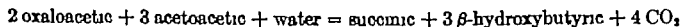
In the presence of kidney brei + barium ions, 80 per cent of the added acetoacetate was converted to citric acid. Thus they isolated in crystal form and identified. The reaction was demonstrated in heart muscle and kidney. Striated muscle was not investigated, but the liver appeared to be unable to bring about the reaction. However, the authors do not exclude the presence of the enzyme from this organ because its high metabolic activity might possibly have oxidized the citric acid away. The precise rôle of barium or magnesium is not clarified. The two hypothetical pro-citric acids should yield upon hydrolysis acetic acid and citric acid, but the authors were unable to demonstrate the presence of the former. To explain this they assume that the acetic acid condenses with another molecule of oxaloacetic to form citric acid. A peculiarity of the condensation reaction, which should be independent of the oxygen tension, is that in air or nitrogen the synthesis of citric acid is very much diminished (1/10) compared to that in oxygen, and the authors supposed that the synthesis must be coupled with some oxidative process. Analyses other than for citric acid were not reported.

On the basis of these experiments, the authors speculated as to the possibility

that all carbohydrate oxidation goes by this mechanism through acetoacetic acid by way of the established pathways  $2 \text{ pyruvic} = \text{acetoacetic} + \text{carbon dioxide}$ . They even suppose that the effective site of insulin action may be upon this enzyme system. In diabetes the mechanism is impaired and acetoacetic accumulates. But this latter hypothesis is hard to defend viz. (a) the liver which is the sole site of ketone formation cannot be demonstrated to contain the enzyme, (b) the muscles—which contain the enzyme—do not produce ketone bodies.

Two equally important papers have appeared opposing the conclusions of Breusch and Wieland. Weil Malherbe (186) cited the following experimental findings: 1, acetoacetate incubated with slices of rat brain or kidney with and without the addition of various members of the citric acid cycle showed insignificant effects on oxygen uptake, formation of citric acid, and disappearance of total ketone bodies; 2, rat brain, testes and kidney equilibrated *anaerobically* with acetoacetate with the addition of oxaloacetic acid showed no significant increase in the disappearance of acetoacetate. The first finding is the precise opposite of the results of Wieland, and the second is covered by the observation of Wieland that the condensation, which should proceed anaerobically, apparently requires oxygen for reasons not apparent at the moment.

The theories of Breusch and Wieland have been even more severely criticized by Krebs and Eggleston (98) in a recent preliminary paper foreshadowing much experimental evidence. The interaction of acetoacetate and oxaloacetate in sheep heart and kidney experiments was studied by a determination of all of the possible reaction products, viz. acetoacetate, oxaloacetate,  $\beta$ -hydroxybutyrate, citrate, isocitrate, *cis*-aconitate,  $\alpha$ -ketoglutarate, succinate, fumarate, malate, pyruvate, lactate. The authors stated that they have shown unequivocally that the extra acetoacetate removed was fully accounted for as  $\beta$ -hydroxybutyrate. They invoked known reactions which they have demonstrated hitherto, viz., the dismutation of malic or  $\alpha$ -ketoglutaric with acetoacetic to form oxaloacetic or succinic together with  $\beta$ -hydroxybutyric acids. Further, oxaloacetic may yield either tricarboxylic (citric, isocitric, *cis*-aconitic) acids,  $\alpha$ -ketoglutaric and malic acids. On this basis they found that their experimental results were readily accounted for by an over all reaction as follows:



They excluded other reactions and stated that "under the condition of our experiments oxaloacetic acid has no effect on the oxidative breakdown of acetoacetic acid. The intermediate stages of this process must still be regarded as obscure."

The ingredients of a sharp controversy are manifest in these four papers and further reports on this new interesting phase of fat metabolism should be of great interest.

These new facts are of far reaching importance and point to a possibility of integration of carbohydrate and fat metabolism in terms of fine chemical detail. However, until confirmation and extension are forthcoming, their full implication must remain a matter of very interesting speculation.

**ACETIC ACIDS** Although acetic acid is readily oxidized in animals, the exact pathway of intermediary metabolism is unknown. Condensation to acetoacetic acid has already been discussed. The possibility that it may be phosphorylated to acetylphosphate before it undergoes oxidation has likewise been considered (Lipman and Perlman, 112). This raises the interesting possibility that it may be formed in the phosphorylated state by beta oxidation of fatty acids. Hence, it cannot readily leave the cell and engages so rapidly in further catabolic reactions that it never accumulates or spills over into the blood, thus explaining the failure to find it in tissues which are actively oxidizing fatty acids. Kleinzeller (90) from studies with tissue slices, concluded that neither glycolic nor glyoxylic acids could be intermediaries in acetic acid oxidation.

The oxidative condensation of acetic acid through the methyl groups into succinic acid has long been proposed (Thunberg, 178), but there is no proof that this reaction occurs in mammalian tissue. However, its occurrence in bacteria has recently been established beyond reasonable doubt (Slade and Werkman, 160).

Toennissen and Brinkman (179), in perfusion experiments with muscles and liver, reported that acetic acid is oxidized by the former and converted to ketone bodies by the latter. Formic acid, which is further oxidized, may be a possible degradation product. They deny the possibility of the formation of succinate from acetic acid in mammalian tissue. The oxidation of acetate, glycolate, and glyoxylate has been studied in guinea-pig kidneys by Kleinzeller (90). Acetic acid is more rapidly oxidized by guinea-pig kidney cortex than by any other tissue examined. The oxidation was inhibited by malonate. Glycolic or glyoxylic acids are not significantly oxidized and hence are excluded as possible intermediates.

A most interesting experiment is that reported by Rittenberg and Bloch (143), using acetic acid with  $C_{13}$  in the carboxyl and deuterium in the methyl group. They fed rats for a period of 8 days with this preparation, and then studied the distribution of the isotopes in the fatty acids of liver and carcass. They concluded from their data that fatty acids are synthesized by successive condensation of  $C_2$  units.

Wieland, Jennin and Schwartz (190) were unable to show any effect of pigeon breast and leg muscle on acetic acid, it is, however, utilized by the kidneys. The aerobic degradation is retarded by malonate. For this reason the authors concluded that the metabolism goes through the succinate acid cycle. Acetic acid was also utilized by liver and lung tissue. The formation of citric acid or  $\alpha$ -ketoglutaric acids could not be demonstrated. Elliot and his co-workers (60) using tissue slices reported acetic acid as utilized by kidney, liver, testis, but not by brain, retina or chick embryo.

Koehler, Hill and Battenwieser (93) studied the oxidation of acetate injected into normal diabetic subjects. It was rapidly oxidized in both instances, but ketone formation as indicated by urinary estimation was relatively small, being somewhat higher in the diabetics.

*Succinic acid* The eagerness of clinicians to seize upon the four carbon dicarboxylic acid as possible therapeutic agents is illustrated by the paper of Koranyi and Szent-Gyorgyi (94). On the basis of the supposed action of succinate in oxidizing away the ketone bodies, they reported beneficial results in the treatment of diabetic ketosis by succinate. The matter was re-investigated by others (51, 117, 177), and the consensus of opinion was that the succinate accomplished no more than an equivalent amount of alkali or glucose.

*Iron.* A possible relation of iron to fat metabolism is indicated by the paper of Elliott and Libet (61) who studied the respiration of tissue suspensions of brain or liver in the presence of small amounts of iron or an iron protein complex obtained from the liver. They found, particularly with added ascorbic acid, a marked influence of iron upon oxygen uptake in the presence of mixed phospholipids (cephalm). The significance of this action of iron upon fat metabolism cannot be evaluated without further experiments.

#### SUMMARY

The intermediary metabolism of fatty acids in mammalian tissue is reviewed under the following heads: 1 Enzyme concerned with fat metabolism 2 Ketone body production site of formation, precursors 3 Chemical mechanisms of ketone production from fatty acids 4 Ketone formation from acetic acid 5 Lower fatty acids 6 Proportion of acetoacetate to  $\beta$ -hydroxybutyrate 7 Lag in ketone production by liver 8 Miscellaneous factors 9 Omega oxidation 10 Utilization of ketone bodies 11 Mode of oxidation of fat in muscles 12 Integration of carbohydrate and fat metabolism 13 Fat to carbohydrate 14 Intermediary pathway of fatty acid derivatives 15 Succinic acid 16 Iron

The author wishes to express grateful appreciation to Dr Benjamin C Riggs, Dr Niels Haugaard and Mrs Barbara Bosworth Pilling for their help in abstracting the literature and compiling the bibliography.

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# THE EFFECTS OF DIETARY DEFICIENCIES UPON THE ORAL STRUCTURES

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The oral tissues are peculiarly sensitive to nutritional deficiencies and dietary aberrations. They are often the first to show the effects of such deficiencies, particularly if the latter are subclinical in nature. The oral cavity mirrors the nutritional status of the body because of two unique characteristics.

1 The oral tissues range from the simplest (mucous membrane) to the most highly specialized tissue (the papillae of the tongue and the enamel and dentin). Hard and soft tissues of both epithelial (enamel) and connective-tissue (dentin and bone) origin may be observed side by side. A highly varied range of responses and types of tissue reactions may thus be seen at any one time. In addition, the oral tissues are constantly subjected to trauma and irritation by mechanical, thermal and bacterial agents and therefore are among the first to exhibit the effects of systemic disturbances.

2 The oral cavity is an internal cavity of the body, dark and moist, and lined by a true mucous membrane which is continuous with the mucosa of the gastrointestinal tract. It receives the secretions of specialized glands and plays an important rôle in digestion. Yet of all the internal cavities of the body, it is the only one that is readily accessible and easily examined without the aid of highly specialized instruments.

The oral tissues have been called the barometer of the state of nutrition of the body. Subclinical states are certainly not rare and from the standpoint of diagnosis, perhaps the most important findings are in the mouth (242). The enamel and dentin are kymographic, fixed records of the past history of the individual. The alveolar bone, the gingivae and the tongue reflect the present internal status of the body accurately and quickly. The routine examination of the teeth, the gums, the tongue, the lips and the saliva at periodic intervals is a simple and fairly accurate method of assessing the nutritional status of both the child and the adult.

The reader is referred to Armstrong (5), Marshall (173), Mellanby (186), and Schour and Massler (229) for earlier reviews on this subject.

**VITAMIN A DEFICIENCY** The classic work of Wolbach and Howe (276) (278) on the dental changes in vitamin A deficiency has been confirmed and extended in the rat (228) (151) (152) (184) (185) (199), in the guinea pig (200) and the dog (150) (189). Since most of our knowledge of the dental effects of vitamin A deficiency is based on findings in the continuously developing incisors of the rat, the effects in this tooth will be described unless otherwise noted.

It has been well established that the function of vitamin A is concerned primarily with epithelial cells and, in these cells, with the process of differentiation (280). Vitamin A deficiency results in a failure of the epithelial cells to differ-

entiate The basal cell layer, therefore, loses its type specificity and tends to produce a stratified keratinized epithelium regardless of the type previously formed In addition, the failure to differentiate allows the basal cell layer to continue its proliferation unchecked Thus a keratinizing metaplasia results in numerous epithelial structures throughout the body (mucous membranes of the trachea, bronchi, conjunctiva, cornea, accessory sinuses, ureter, vagina, uterus, and periurethral glands as well as the skin) In the developing tooth germ, the first effect of vitamin A deficiency is found in the highly specialized odontogenic epithelium responsible for enamel formation and guiding the formation of dentin

*Odontogenic epithelium* The primary effect is seen as a failure of the young cells of the odontogenic epithelium to undergo normal histo-differentiation and morpho-differentiation (228) This results in a persistence and an increased rate of cell proliferation which in turn leads to an invasion of proliferating epithelial cells into the pulp This epithelial invasion is very characteristic of vitamin A deficiency (228)

In young rats whose mothers were on a vitamin A deficient diet for five months preceding their birth, the changes are more severe and include a distortion in the shapes of both the incisors and molars (184) (185)

*Growing enamel* The mature enamel forming cells of the odontogenic epithelium (ameloblasts) become small in size and disturbed in function so that the enamel matrix is arrested in its formation and calcification Enamel hypoplasia is therefore a common finding in the rat following prolonged vitamin A deficiency

The normal orange pigment of the enamel, which is the final product of the ameloblasts, is lacking and the tooth has a paper white, unglazed appearance (120) (236)

*Growing dentin* One of the functions of the odontogenic epithelium is to organize and cause the differentiation of the subjacent mesenchymal cells of the pulp into odontoblasts In spite of the fact that vitamin A deficiency affects epithelial cells primarily, the first recognizable histologic change is seen in the mesenchymal odontoblasts Morphologic changes in the ameloblasts occur much later although their function is affected before the odontoblastic changes are observed

The odontoblasts on the lingual (cementum-covered) portion of the incisor show an early atrophy and depolarization (278) As a result the growth of the cementum-covered dentin is seriously affected It is atypical in structure, lacks the normal tubular arrangement and contains cellular and vascular inclusions Pohto (199) has emphasized the peg like dentin projections into the pulp and the prominent foldings.

On the labial (enamel-covered) side of the incisor, the odontoblasts normally differentiate under the influence of the ameloblasts rather than under the less differentiated odontogenic epithelium. The odontoblasts on this side of the tooth appear to be normal and continue to lay down normal dentin long after the odontoblasts on the lingual have disappeared In fact, the labial dentin becomes excessively wide in contrast to the abnormally thin lingual portion

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**VITAMIN A DEFICIENCY** The classic work of Wolbach and Howe (276) (278) on the dental changes in vitamin A deficiency has been confirmed and extended in the rat (228) (151) (152) (184) (185) (199), in the guinea pig (200) and the dog (150) (189). Since most of our knowledge of the dental effects of vitamin A deficiency is based on findings in the continuously developing incisors of the rat, the effects in this tooth will be described unless otherwise noted.

It has been well established that the function of vitamin A is concerned primarily with epithelial cells and, in these cells, with the process of differentiation (280). Vitamin A deficiency results in a failure of the epithelial cells to differ-

entiate The basal cell layer, therefore, loses its type specificity and tends to produce a stratified keratinized epithelium regardless of the type previously formed In addition, the failure to differentiate allows the basal cell layer to continue its proliferation unchecked Thus a keratinizing metaplasia results in numerous epithelial structures throughout the body (mucous membranes of the trachea, bronchi, conjunctiva, cornea, accessory sinuses, ureter, vagina, uterus, and perineal glands as well as the skin) In the developing tooth germ, the first effect of vitamin A deficiency is found in the highly specialized odontogenic epithelium responsible for enamel formation and guiding the formation of dentin

*Odontogenic epithelium* The primary effect is seen as a failure of the young cells of the odontogenic epithelium to undergo normal histo-differentiation and morpho-differentiation (228) This results in a persistence and an increased rate of cell proliferation which in turn leads to an invasion of proliferating epithelial cells into the pulp This epithelial invasion is very characteristic of vitamin A deficiency (228)

In young rats whose mothers were on a vitamin A deficient diet for five months preceding their birth, the changes are more severe and include a distortion in the shapes of both the incisors and molars (184) (185)

*Growing enamel* The mature enamel forming cells of the odontogenic epithelium (ameloblasts) become small in size and disturbed in function so that the enamel matrix is arrested in its formation and calcification Enamel hypoplasia is therefore a common finding in the rat following prolonged vitamin A deficiency

The normal orange pigment of the enamel, which is the final product of the ameloblasts, is lacking and the tooth has a paper white, unglazed appearance (120) (236)

*Growing dentin* One of the functions of the odontogenic epithelium is to organize and cause the differentiation of the subjacent mesenchymal cells of the pulp into odontoblasts In spite of the fact that vitamin A deficiency affects epithelial cells primarily, the first recognizable histologic change is seen in the mesenchymal odontoblasts Morphologic changes in the ameloblasts occur much later although their function is affected before the odontoblastic changes are observed.

The odontoblasts on the lingual (cementum-covered) portion of the incisor show an early atrophy and depolarization (278) As a result, the growth of the cementum-covered dentin is seriously affected It is atypical in structure, lacks the normal tubular arrangement and contains cellular and vascular inclusions Pohto (199) has emphasized the peg like dentin projections into the pulp and the prominent foldings

On the labial (enamel-covered) side of the incisor, the odontoblasts normally differentiate under the influence of the ameloblasts rather than under the less differentiated odontogenic epithelium The odontoblasts on this side of the tooth appear to be normal and continue to lay down normal dentin long after the odontoblasts on the lingual have disappeared In fact the labial dentin becomes excessively wide in contrast to the abnormally thin lingual portion



The reason for this difference may be a selective alteration in the rate of dentin apposition. Instead of the normal rate of 16 microns per day, vital injections with Alizarine Red S show that the enamel-covered dentin shows an accelerated rate (up to 19 microns per day) and the cementum-covered dentin shows a corresponding *decelerated* rate (down to 6 microns per day) (228).

*Pulp* The invasion of the pulp by odontogenic epithelium is very characteristic of a chronic vitamin A deficiency. Amorphous dentin may be formed in the pulp as a result of the organizing influence of this aberrant epithelium upon the invaded pulpal mesenchyme.

*Calcification of enamel and dentin* The enamel of the incisors appears dull white and opaque, while the incisal edge is short and blunt, indicating a deficient calcification of the enamel (236).

The dentin shows an interglobular texture. King (151) pointed out that the calcification of the dentin was disturbed in his animals in spite of the fact that they were given ample quantities of vitamin D, calcium and phosphorus. The super-position of a vitamin D deficiency upon a basic diet deficient in vitamin A increases the disturbance in dentin calcification (125) (187) (228) (278).

Vital staining with Alizarine Red S produces a faint red color instead of the normally deep red (228). The teeth contain a lower total ash and a slightly lower percentage of phosphorus but a higher percentage of calcium in the ash (236). All these facts point to a deficient calcification of both the enamel and dentin in vitamin A deficiency.

*Alveolar Bone* Vital injections of Alizarine Red S indicate that the alveolar bone is retarded in its rate of formation (228). In the dog, there is an increased density of the alveolar bone with roentgenographic evidence of an ill-defined lamina dura (150).

The cementum is thickened. The periodontal membrane is irregular in width and abnormally enlarged in the fundic region of the rat incisor (228).

*Eruption* Eruption is retarded (236) and ceases entirely in prolonged deficiencies (228). In the dog, the teeth are also malposed and show malformed roots (150).

*Gingivae* In both the dog and the rat the gingival epithelium becomes hyperplastic and shows a marked keratinization (152) (185) (188) (187). The tissue is easily invaded by bacteria, leading eventually to periodontal disease. In prolonged deficiencies, deposits of calculus occur on the necks of the teeth (152).

*Salivary glands* The major and minor salivary glands undergo the typical keratinizing metaplasia that characterizes all the epithelial cells in vitamin A deficiency (280). So far as we could ascertain, an analysis of changes in the quality of the saliva has not been made, although such studies would help clarify the relation of vitamin A deficiency to dental disease.

*Other oral changes* Green and Mellanby (102) observed abscesses in the floor of the mouth and suppurating glands in the neck in 72 to 90 per cent of 92 rats deficient in vitamin A.

*Effects of Replacement Therapy* *Cellular* Wolbach and Howe (278) found effects within seven days after feeding butter-fat. The odontogenic

epithelium and the odontoblasts resume their normal form and function. The ectopic epithelial cells in the pulp do not differentiate sufficiently to become ameloblasts which might form enamel but regain their organizing influence over the adjacent mesenchymal cells which differentiate promptly into odontoblasts and form dentin (228). Excessive local formations of atypical dentin within the pulp thus increase with recovery. Morphological recovery of the cells is complete within 19 days following replacement therapy (278).

**Pigment** Irving and Richards (120) noted changes in enamel pigmentation which varied with different doses of vitamin A replacement.

**Rate of growth** Measurements of the rate of dentin apposition by means of vital staining with Alizarine Red S show that the deviation from the normal rate of growth is in direct proportion to the severity and duration of the vitamin A deficiency. The rate of apposition also varies with the amount of replacement with vitamin A. The use of this sensitive dentin reaction as a biologic method of measuring the vitamin A content of foods has therefore been suggested (224).

**Tumor formations** Long-continued, chronic vitamin A deficiency is made possible by intermittent feeding of minimal quantities of vitamin A. Orten and Smith (43) observed tumor like formations (odontomas) in and about the incisors in one-third of their experimental animals. These tumors arise as a result of the scattering of portions of the odontogenic epithelium into the surrounding soft tissues of the jaws. During the feeding intervals these scattered epithelial transplants organize the adjacent mesenchymal cells to form dentin and therefore also odontomas.

**Vitamin A Deficiency in Man Enamel hypoplasia** Vitamin A deficiency may cause a disturbance in the developing tooth germ only in children under six years of age, since after that time the crowns of all the teeth (with the exception of the third molars) are completed. Boyle (31) described changes in the enamel organ of a tooth germ of a human infant suffering from vitamin A deficiency. The changes were similar to those found in the rodent incisor. However, the suggestion that vitamin A deficiency is a common cause of enamel hypoplasia is not supported by findings other than the single case reported by Boyle (31). Bloch (26) (27) studied a number of children suffering from severe xerophthalmia and blindness caused by vitamin A deficiency. He found no dental changes. Investigation on the cause of enamel hypoplasias also does not support the assumption that vitamin A deficiency is a common cause. In fact, in sixty cases of enamel hypoplasia investigated by Sarnat and Schour (216) not a single case of enamel hypoplasia was found to be associated with a vitamin A deficiency.

**Oral epithelium** In the adult, the possible effect of vitamin A deficiency is limited to the oral epithelium. Vitamin A deficiency may result in hyperkeratotic changes in the oral mucosa similar to those found on other mucous surfaces. Abels et al (1) suggest a possible relationship between vitamin A deficiency and hyperkeratosis of the gingivae and oral leukoplakia. Hyperkeratosis of the gingivae has been interpreted clinically as resulting from vitamin A deficiency.

**Caries** There is no definite evidence to show that caries is increased because

of a deficiency in vitamin A (60) Administration of supplements of vitamins A and D to children for a period of over one year had no significant effect on caries progress (57) However, the diminished production of lysozyme in most of the body fluids, with resulting loss of bacteriostatic action, may also occur in the saliva (70) The possible rôle of this factor in the saliva as a contributory cause in caries deserves investigation

*Hypervitaminosis A* Significant dental changes could not be found in experimental hypervitaminosis A (280)

**VITAMIN B DEFICIENCIES** Unlike the oral findings in vitamin A deficiency which are derived largely from animal experimentation, those in vitamin B deficiencies are for the most part based on clinical observations in man For this reason, the mechanism of action and many details of the histologic changes in vitamin B deficiencies still remain to be elucidated

The effects of deficiencies of vitamins A, C and D, and of the minerals, are concerned chiefly with tooth and with bone development However, deficiencies in the B vitamins are concerned primarily with the oral soft tissues (gingivae, tongue and mucous membranes)

*Thiamine (Vitamin B<sub>1</sub>) Deficiency* Thiamine deficiency in man is characterized by a marked sensitivity of the oral tissues (171) Weisberger (268) observed small, pinpoint vesicles resembling herpetic lesions on the buccal mucosa, under the tongue or on the palate in elderly patients who were deficient in thiamine These lesions healed after thiamine therapy No typical glossitis was observed in experimental subjects studied by Williams et al (275)

Govier and Greig (100) observed necrotic erosions in thiamine-deficient dogs However, these lesions healed only after vitamin C was added to the vitamin B replacement There is therefore reason to question whether these lesions were caused by the thiamine deficiency The experimental and clinical effects of thiamine deficiency upon the oral tissues need further elucidation

*Riboflavin (Vitamin B<sub>2</sub>) Deficiency* Ariboflavinosis is characterized by (1) a magenta-colored glossitis, (2) cheilosis, (3) capillary dilatation and proliferation on the cornea of the eyes, and (4) a seborrheic dermatitis of the naso-labial folds, alae nasi, eyelids and ears (246) (247) When the quartet of these symptoms occurs, the clinical diagnosis of ariboflavinosis is not difficult

Ariboflavinosis shows early in its course a characteristic magenta-colored, pebbly glossitis which is distinguishable from the scarlet-red, smooth, atrophic glossitis of pellagra and which disappears only after riboflavin therapy (162) (129) (130) (268) (246) Ariboflavinosis also presents a characteristic cheilosis Sebrell and Butler (232) conducted an experimental study on 18 healthy women placed on a riboflavin-deficient diet After 94 to 130 days, thirteen developed a fissuring and maceration of the angles of the mouth (cheilosis) resembling perlèche The lesions responded to additions of riboflavin to the diet but not of nicotinic acid These findings have been confirmed repeatedly (214) (131) (133) Jones et al (133) made a careful slit-lamp examination and analysis of the characteristic stomatitis caused by riboflavin deficiency in 1746 men in an African army camp The stomatitis consisted essentially of a painful glossitis

(not magenta colored) and a painful cheilosis with excessive salivation. The lesions responded to additions of riboflavin or fresh yeast to the diet but not to additions of nicotinic acid.

While the exact mechanism involved in the production of both the glossitis and the cheilosis is somewhat obscure, the clinical stages have been clearly described and are readily recognized.

**Glossitis.** The glossitis begins in mild deficiencies with a soreness at the tips and/or lateral margins of the tongue. These sites become reddened and glazed as a result of the atrophy of the filiform papillae. The fungiform papillae are engorged and flattened (mushroom shaped), giving the tongue a coarsely granular or pebbly texture. The lesions extend backward over the dorsum of the tongue in the form of ovoid patchy areas of desquamation. In moderate chronic deficiencies, the entire tongue presents the characteristic granular glossitis. In very severe cases, all traces of the lingual papillae disappear, leaving the tongue smooth, shiny and glazed. Fissures frequently develop. The tongue becomes atrophic with sharp margins indented by the teeth.

**Cheilosis.** The cheilosis begins as a tiny, raw, red, painful area at the commissure of the lips, at the muco-cutaneous junction. The area becomes larger and is soon covered with a white, adherent epithelial membrane. In severe cases, multiple fissures appear which are very painful. The lesion tends to spread to the lower lip causing a fissuring and cheilitis. It may also spread over the skin but characteristically spares the upper lip. The gingivae are not affected.

Recent findings indicate that the cheilosis may not necessarily be specific for ariboflavinosis but may depend somewhat upon concurrent deficiencies in pyridoxine (238) (169), nicotinic acid (115) or the entire B-complex (129) (130).

**Pseudo-ariboflavinosis.** Clinically, the glossitis and cheilosis of ariboflavinosis must be distinguished from the atrophic glossitis and pseudo-cheilosis resulting from a closed bite in edentulous persons or in those with poorly fitting artificial dentures (71) (268) (196). Neither should traumatic fissures or monilial infections at the angles of the mouth (perlèche) be mistaken for the cheilosis of ariboflavinosis (244) (130). Sutton and Sutton (245) suggest a causal relationship between cheilosis and perlèche. Although perlèche is a specific monilial infection, it may superpose or follow an underlying ariboflavinosis just as a Vincent's infection may follow and superpose a pellagrous stomatitis.

**Effect on bone formation.** A possible rôle of riboflavin in skeletal development has been pointed out by Warkany and Schraffenberger (257) who found many congenital malformations, including cleft palate and impaired development of the mandible, in new-born rats whose mothers were subjected to riboflavin deficiency. In a recent abstract, Ross (215) reports a severe type of alveolar-crest atrophy and loosening of the incisors in young adult males suffering from riboflavin deficiency. The rôle of riboflavin in bone growth, however, awaits further investigation.

**Vitamin B<sub>2</sub> deficiency in monkeys.** Experimental multiple vitamin deficiencies in monkeys produced lesions of the gingivae, the periodontal tissues and the oral mucosa. The most severe changes (including noma) occurred in deficiencies of vitamin B<sub>2</sub> complex (251) (249) (47).

*Niacin Deficiency Glossitis* Niacin deficiency is characterized by a typical, painful, fiery-red glossitis which shows a spectacular and prompt disappearance after administration of nicotinic acid or its amide (144) In the prodromal stage of niacin deficiency, the patients complain of weakness, lassitude, insomnia, headache, anorexia and loss of weight (171) They also complain of a burning sensation in the tongue (glossodynia) and oral mucosa The tongue is swollen and reddened at the tip and lateral margins and indented by the teeth The lingual papillae are red and hypertrophied at this stage, giving the tongue a reddened appearance Kirkland (154) points out that few pellagrins fail to give a history of prodromal painful glossitis, gingivitis and stomatitis Kruse (163) has described minutely the lingual manifestations as observed with the aid of the biomicroscope in very mild cases of aniacinosis None of his cases showed skin lesions

In the acute stages of niacin deficiency, the entire oral mucosa becomes very vascular and fiery red The tongue becomes tender and painful and the entire mouth feels painfully scalded There is excessive salivation In the more advanced cases, the lingual papillae completely disappear, leaving the tongue smooth, dry and glazed The tongue literally sheds its epithelium, beginning at the tip and lateral margins

*Gingivitis* In contrast to riboflavin deficiency, the gums and gingivae are also attacked One of the earliest prodromal symptoms of a niacin deficiency is a painful gingivitis and stomatitis Beginning with the interdental papillae and progressing very rapidly and extensively, the gingivae become very tender and painful and often become ulcerated The ulcerations usually present a thick, grayish exudate or membrane which gives rich cultures of Vincent's organisms, and may spread to the entire oral mucosa The Vincent's infection appears in direct proportion to the severity of the deficiency and subsides after adequate niacin therapy (153) King (153) found that the most effective treatment is a combination of niacin and local hygienic therapy

*Dermatitis* In persons with these oral symptoms, the presence of a characteristic dermatitis (confluent red-brown areas with sharp margins, bilateral symmetry and keratosis) is ample evidence of a niacin deficiency (154)

King (153) and Kaufman (144) have shown that an atypical pellagra (pellagra sine pellagra) may occur The symptoms in these cases are neither as severe nor as constant as in the typical pellagra and consist principally of vague neuropsychiatric complaints and a relatively mild stomatitis with an atrophic burning glossitis (glossodynia) (172) (50) The symptoms disappear after niacin therapy

*Secondary pellagra* Although the typical pellagra is not common in the northern states (74), secondary pellagra is rather common and widespread (144) (10) Loss of teeth, partial or complete, dental infections, periodontal disease, tooth extraction, fractures and tumors of the jaws and painful stomatitis are frequently the cause of a secondary pellagra Such patients are frequently obliged to substitute a soft diet consisting mainly of soups, mush and cooked cereals instead of a balanced one containing meats and uncooked vegetables (10)

*Experimental niacin deficiency* Black tongue has been found among dogs

owned by families suffering from pellagra, since these animals live on scraps of the same food their masters eat (241). This condition consists of a glossitis and stomatitis similar to that found in human pellagra. The stomatitis tends to be severe and ulcerative and is quickly cured by niacin (72). The lesions in the mouth often appear before the other general symptoms (66) (70), and appear first as areas of redness on the floor of the mouth, the cheeks or along the inner side of the upper lip. As the deficiency becomes more severe, these areas become dark red and are finally covered with a greenish-gray membrane, due to a superficial necrosis of the oral epithelium. Once the necrosis begins, fetor ex ore and drooling appear. The mouth may become entirely covered with pustules and so foul as to resemble rotten meat (48).

*Pyridoxine (Vitamin B<sub>6</sub>) Deficiency* Rosenblum and Jolliffe (214) have described a specific edematous magenta-colored glossitis which responds only to pyridoxine therapy. However, the possibility of a concurrent riboflavin deficiency has been considered.

*Pantothenic Acid Deficiency* Becks and Morgan (17) and Becks, Wainwright and Morgan (19) describe the effects in dogs of a deficiency of the entire filtrate fraction of the vitamin B complex, which they consider to contain pantothenic acid plus one or more unknown essential elements. The deficiency leads to severe gingival inflammation, necrosis and finally a desquamation of the oral epithelium as well as an osteoporosis and progressive marginal atrophy of the alveolar bone. The supporting spongiosa is porotic and replaced by fat tissue. The clinical and histologic appearance of the alveolar atrophy resembles the atrophic type of periodontosis observed in man. However, the authors caution against drawing generalized conclusions since only one or two animals were used in each experiment and since man may not react as does the dog. Becks and his co-workers feel that all these changes are not caused by a deficiency in pantothenic acid alone since the addition of crystalline pantothenic acid to the diet deficient in the entire filtrate factor did not completely protect the oral tissues against pathologic changes.

Ziskin et al (286) observed oral lesions consisting of fissures and crusting at the angles of the mouth and ulcers on the buccal mucous membranes and on the tongue in rats on a low pantothenic acid diet supplemented with additions of zinc carbonate.

*Vitamin B Deficiency and Oral Disease* Caries Kniesner, Mann and Spies (159) found a low incidence of dental decay in pellagrins. This is true in spite of the generally poor oral hygiene and high carbohydrate diets. The fact that thiamine is essential to bacterial growth and carbohydrate metabolism may explain the inverse relationship between vitamin B complex deficiency and dental decay.

*Periodontal disease* Although the incidence of caries was found to be very low in pellagrins, that of periodontal disease is very high (171) (10).

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also point out that the lesions of avitaminosis B are not confined to the mouth, but affect the entire gastro-intestinal tract and therefore may also play a rôle in the etiology of gastric and intestinal cancer

*Glossodynia* A measurable proportion of patients in the older age groups who complain of abnormal oral sensations (glossodynia and abnormal taste sensations) respond favorably to therapy with the vitamin B complex. Occasional cases respond specifically to niacin or to thiamine therapy. However, in man deficiencies are usually multiple and rarely single

*Vincent's infection* The relation between vitamin B deficiency, more particularly niacin deficiency, and Vincent's infection of the gingivae and other oral tissues, has often been noted (153). Vincent's infection often follows a niacin deficiency but a niacin deficiency is only one of many systemic conditions which predispose to Vincent's infection

*Herpetic infections* Gerstenberger (96) and Burket and Hickman (42) report a prompt and often dramatic response of herpetic lesions to vitamin B complex therapy. In some instances thiamine chloride alone was effective (42). Weisberger (268) observed herpetic-like lesions in thiamine-deficient patients

Herpetic lesions (herpes labialis, solitary and recurrent aphthae, herpetic stomatitis and herpes genitalis) are specific infections caused by the herpes simplex virus and, like Vincent's infections, are not a specific symptom of vitamin B deficiency. Therefore not all cases will be improved by vitamin therapy (42). The evidence indicates, however, that one of the important predisposing factors in many cases of both Vincent's and herpetic infections is an underlying vitamin B deficiency

**VITAMIN C DEFICIENCY** Vitamin C seems to play a primary rôle in the differentiation of connective-tissue cells, and therefore in the capacity of these cells to form and maintain intercellular substance (8) (277). Those oral tissues which are mesenchymal products (dentin, bone, periodontal membrane) or whose integrity depends particularly upon their vascular supply (gingivae) are primarily affected. The dental changes described below apply largely to the continuously developing incisors of the guinea pig, which has been the animal of choice in studies of scurvy (277) (37) (108). The periodontal changes have been described in man (40) (272) (273), in monkey (250) (251) and in the guinea pig (37)

*Pulp and growing dentin* The earliest dental effect is a disturbance in the histo-differentiation of the odontoblasts. These cells atrophy, and become disorientated and are disturbed in function in even mild deficiency states. The vessels of the pulp are dilated and hemorrhagic

The characteristic disability of the mesenchymal cells to form intercellular substances results in an amorphous and irregular dentin which has been variously called osteodentin, pulp bone and calcific scar (79). The dentinal tubules are few in number and irregularly arranged. Atrophic cells of the pulp may become enclosed in the matrix, simulating the secondary dentin in man. Eventually, the dentin stops forming and as a result the predentin (the newly deposited layer next to the pulp) becomes hypercalcified. This apparent overcalcification of the predentin is secondary to the arrest in dentin formation (280)

Eventually, the odontoblasts become morphologically indistinguishable from other pulpal cells (34). Boyle, Bessey and Howe (37) have pointed out that these changes are not unlike those normally found in the oldest or incisal portions of the continuously growing incisor tooth where the odontoblasts have completed their life-span and undergone senile atrophy. However, in vitamin C deficiency these regressive cellular changes are greater in intensity and occur at the younger, formative end of the incisor.

The histologic changes mentioned above are consistent and so characteristic in their sequence that histologic examination and grading of these changes have provided a biological method for the assay of vitamin C (112) (147).

Fish and Harris (77) believe that the dental changes are the direct result of a primary premature degeneration of the odontoblasts as well as the ameloblasts. They regard the calcified predentin and calcific deposits in the pulp as secondary effects and call them calcific scar tissues. They claim that whenever the odontoblasts degenerate and lose their dentinal fibrils, the pulp attempts to seal off the dead primary dentin by such calcific scar tissue.

*Rate of dentin apposition* Boyle, Bessey and Howe (37) found, by means of vital staining with Alizarine Red S, that dentin formation is retarded and eventually ceases in advanced deficiencies.

*Enamel epithelium and growing enamel* Atrophic changes in the enamel epithelium and enamel hypoplasia appear considerably later than do the changes in the odontoblasts (33). Boyle (33) points out that the atrophy of the enamel epithelium is caused by traumatic injuries to the enamel organ. The lack of collagen fiber formation in the periodontal membrane weakens that structure on the lingual surface of the tooth and leads to a failure to resist incisal stresses so that injury to the enamel epithelium on the labial surface results. This is in contrast to the views of Fish and Harris (77) who believe that the atrophy of the enamel epithelium is caused directly by the vitamin C deficiency.

*Alveolar bone and periodontal membrane* The osteoblasts lose their ability to form bone and the fibroblasts to form collagenous fibers. This results in a weakening of the structures supporting the tooth and in a porosis and an atrophy of the alveolar bone which is replaced by connective tissue (272) (104).

Occlusal stresses are normally resisted by the fibers of the periodontal membrane. In vitamin C deficiency, the periodontal fibers rupture easily, with the result that the tooth impinges upon the weakened, atrophic alveolar bone and causes its resorption. The teeth thus lose their ability to withstand normal occlusal stresses, become loose and may be easily extracted (35). These changes have been considered to be similar to those characteristic of systemic periodontosis (diffuse alveolar atrophy) in man (35) (40).

*Gingivae* In the continuously growing incisors and molars of the guinea pig the periodontal tissues are replaced very rapidly so that the scorbutic effects are usually not as outstanding as they are in monkeys and in man. In addition, the striking changes in the pulp and growing dentin have diverted attention from the changes in the periodontal tissues. Boyle, Bessey and Wolbach (35) observed that the gingivae of the scorbutic guinea pig were congested but no pocket formations

Westin and Kalnins (274) succeeded in producing scurvy in the rat in spite of the ability of this animal to synthesize vitamin C. They describe severe disturbances in the periodontal tissues of both the continuously growing incisors and the molars which, like those in man, are of limited growth. They observed a severe marginal gingivo-alveolar osteitis and osteomyelitis, the extent and severity of which were in direct proportion to the length of the experimental period. In 162 days of vitamin C deficiency, the alveolar process around the molars was completely destroyed and the teeth were lost. The changes in the incisors, because of their continuous growth and development, were much less severe.

*Eruption* Dalldorf and Zall (54) have reported retardation in the eruption of the incisors of guinea pigs. They and others (69) suggest the use of the rate of eruption as an accurate index of the degree of scurvy.

*Changes in monkeys* Tomlinson (250) observed a marked hyperemia and hypertrophy of the gingivae in four vitamin-C-deficient and four vitamin-C and calcium-deficient monkeys. The free margin of the gingivae became so hypertrophied as to cover the entire crown of the teeth. Food debris tended to accumulate and produced a foul odor. In severe cases, areas of the free gingivae became necrotic and the teeth became loose. Subperiosteal hemorrhages lifted the soft tissue from the underlying bone.

The hemorrhage and necrosis extended for a short distance into the periodontal membrane which was markedly deficient in fibers. The alveolar bone was thin and showed many subperiosteal hemorrhages and abnormal resorptions but no necrosis. The bone marrow was edematous and contained an increased number of young fibroblasts but was markedly deficient in fibers. Extensive hemorrhages also occurred in the joints, including the temporo-mandibular joint, and in the growing epiphyseal plates, causing the latter to separate and eventually disappear.

Those animals which were placed on a diet deficient in both vitamin C and calcium showed less severe changes than those deficient in vitamin C alone. Vitamin C therapy relieved the earlier symptoms but there was no recovery of the interdental papillae or other tissues which had advanced to the stage of necrosis, nor was there a tendency to reattachment of the separated gingivae (86) (87).

*Factors Influencing the Effects of Vitamin C Deficiency* *Increased stress* Dalldorf and Zall (54) observed more advanced changes when the teeth of vitamin C deficient animals were under increased occlusal stress. This factor is particularly important in man.

*Pregnancy* Scorbutic guinea pigs show more pronounced changes during pregnancy. The effects are more severe in the continuously growing teeth of the mother than in the young (56). The relation of vitamin C deficiency to pregnancy in man has not been adequately studied.

*Toxins* King et al (148) found that the administration of diphtheria toxin to guinea pigs caused marked injury to the odontoblasts and to dentin formation. Administration of 50 mgm of ascorbic acid per day protected against this injury.

The effect of toxic states in man in the production of dental and oral lesions has been noted often but has not been adequately studied, particularly in relation to increased vitamin C needs

*Replacement therapy* Boyle, Wolbach and Bessey (34) observed that the pulpal cells differentiated promptly after even partial replacement therapy. However, instead of the normal dentin deposited in normal incremental layers arranged parallel with the pulpal surface, an irregular, bone-like dentin is deposited in the pulp in the form of long spicules which project toward the center of the pulp cavity. These spicules represent a partial recovery of the dentin forming cells to form dentin. Where the odontoblasts had survived the vitamin C deficiency and the replacement was adequate, dentin formation became normal in structure and proceeded at a normal rate (34).

Boyle, Bessey and Howe (37) found that in vitamin C deficient guinea pigs, the amount of dentin deposited in the formative end of the incisor varies directly with the amount of ascorbic acid administered. They therefore suggest the possibility of utilizing this reaction as an objective, biologic assay for the determination of vitamin C content in foods.

*Vitamin C Deficiency in Man Growing teeth.* Boyle (32) examined the teeth of two scorbutic infants and found none of the changes described in the growing dental tissues of a scorbutic guinea pig. One infant showed small cysts in the enamel organ and minute hemorrhages. Boyle (32) explains the absence of the characteristic changes on the basis that human teeth grow much slower and that unerupted teeth are free from functional stresses. There may of course be a basic difference in the reaction of human and other species. There has been no human demonstration of the changes observed in the growing dental tissues in the guinea pig (279).

*Eruption.* Pediatric texts frequently state that the eruption of teeth is delayed in scorbutic infants. In our experience, scorbutic infants show an accelerated eruption of the teeth.

*Adult teeth.* Westin (272) (273) made a detailed and comprehensive histopathologic study of 18 human adults suffering from various degrees of scurvy. The enamel showed no changes. The pulp was hyperemic and edematous and contained various formations of secondary dentin. Calcified or necrotic areas were also present. The odontoblasts showed degeneration. The dentinal tubules underwent porotic changes which Westin regards as dentinolysis.

*Gingivae.* The effects of vitamin C deficiency in man are confined largely to the continuously growing periodontal tissues and rarely affect the enamel or dentin. When the teeth are not yet erupted or have been extracted, the marginal gingivae, the alveolar crest and the organized periodontal membrane are non-existent and therefore the effects on the periodontal tissues are absent. Unlike vitamin B deficiencies, scurvy rarely affects the tongue, lips or buccal mucosa.

*Scorbutic gingivitis.* The first clinical evidence of latent scurvy in man is seen as a hyperemia and swelling of the interdental and marginal gingivae which become very tender and bleed upon the most trifling trauma (164).

In mild acute cases the subsurface capillaries become engorged and dilated so

that the gingivae become violently reddened and in moderate cases swollen, giving the surface a smooth, shiny, dark-red appearance. The process begins on the interdental papillae and soon involves the marginal (free) gingivae, forming a swollen collar around the teeth which bleeds very easily upon slight trauma and is sharply demarcated from the purplish alveolar (attached) gingivae. The gingival crevice is enlarged to form a pocket which soon is filled with debris and calculus and which in turn irritates the gingivae and aggravates the condition.

In fully developed acute scurvy, the gingivae become boggy and are soon denuded of epithelium, showing dilated, thin-walled, easily ruptured blood vessels. The color of the gingivae changes rapidly to a violaceous red. The alveolar (attached) gingivae may also become involved until the gums are very much swollen. In infants, the swollen tissue may entirely cover the newly erupted deciduous teeth. In severe acute cases the hemorrhage tends to be spontaneous and the pain is constant and intense. The gingivae may become ulcerated and secondarily infected with Vincent's organisms.

In mild chronic scurvy, the subsurface capillaries undergo relatively slight dilatation and engorgement. The interdental papillae and the marginal gingivae become swollen and edematous but not reddened. Atrophy of the marginal gingivae is very marked so that they tend to fall away from the teeth. This results in gingival pockets and the accumulation of debris from which a distinctly foul odor emanates. The marginal gingivae form a spongy and edematous collar which tends to recede from the necks of the teeth.

In severe chronic cases, the swollen gingival collar may become covered with small ulcers and granulation tissue. At the same time, hemorrhages and swelling of the periodontal membrane occur and are followed by rarefaction and loss of the alveolar bone. As a result, the teeth soon become loosened and fall out.

Kruse (164) gives an excellent and detailed description of the above changes in acute and chronic scorbutic gingivitis as seen with the aid of the biomicroscope.

Local factors, particularly trauma, calculus and malhygiene, undoubtedly play a significant rôle in the progress of the scorbutic gingivitis and the periodontal breakdown, since these lesions begin and are obvious only at sites of irritation and stress. They do not occur at all if the teeth are unerupted or absent. A similar situation obtains in other types of systemic gingivitis and periodontal disease, such as lead lines, diabetic gingivitis, etc. (231).

*Vitamin C Deficiency and Dental Disease* *Gingivitis* Gingivitis is a very common disease which affects more than 75 per cent of all adults to some degree. It is caused for the most part by local factors such as malhygiene, calculus and trauma. However, in view of the facts that vitamin C deficiency predisposes to gingival disease and that latent scurvy shows a high prevalence (30-65 per cent) among persons who are on an apparently adequate diet (283), the possible systemic rôle of vitamin C deficiency in gingivitis cannot be neglected.

While vitamin C deficiency has frequently been cited as an underlying cause in gingivitis (164) (44) (28) (243), there are many investigators who found no correlation between low blood plasma levels of ascorbic acid and the average case of gingivitis (202) (204) (203) (107) and many who obtained no therapeutic

results from large oral doses of vitamin C (59) Moreover, in spite of the recognized increase in vitamin C intake in the United States, there has been no apparent decrease in the incidence of gingivitis (201) On the other hand, when the gingivitis is caused by a latent or subclinical but demonstrable vitamin C deficiency, excellent results follow treatment with ascorbic acid (205), (243) (145)

Additional and better controlled investigations are needed to supply conclusive evidence on this important but controversial problem

*Vincent's infection and scorbutic gingivitis* A secondary Vincent's infection maybe superposed upon an underlying scorbutic gingivitis (243) (145) The scorbutic gingivitis is distinguished from a primary Vincent's infection by the fact that (a) the ulcers are small and localized to the marginal gingivae, (b) there is no lymphadenopathy, and (c) there is a dramatic response to vitamin C therapy (145)

*Periodontal disease (alveolar bone and periodontal membrane)* The possibility of vitamin C deficiency as the etiologic agent in periodontal disease has been pointed out many times (40) (272) (273) (28) (46) (267) (35) (36) (110) However, vitamin C therapy in patients suffering from periodontal disease has not been attended with conspicuous success (202) (44)

Boyle, Bessey and Wolbach (35) point out the similarity in the histopathology of the attachment apparatus between both experimental and clinical scurvy and diffuse alveolar atrophy Westin (272) (273) describes the changes in the periodontal tissues in human scurvy as 1, marked widening of the periodontal space, 2, resorption of the cementum and alveolar bone, 3, hemorrhage, 4, osteoporosis and disappearance of hematopoietic tissue There is no pocket formation

Weisberger et al (267) reported direct correlation between low blood ascorbic acid levels and periodontal disease Therefore Boyle, Bessey and Wolbach (35) recommended blood tests for ascorbic acid in all cases of periodontal disease However, negative findings have also been reported (44), (202)

In a study by Crandon, Lund and Dill (53) one of the authors subjected himself to vitamin C deficiency and found that the earliest changes that could be observed were in the dental roentgenograms which showed slight but definite interruptions in the lamina dura (alveolar bone proper) These authors therefore suggest the possibility that roentgenographic observation of the lamina dura be used as a criterion in the diagnosis of incipient scurvy

There is no doubt that vitamin C is as necessary for the maintenance of periodontal tissue as it is for rapid wound healing However, although the relation of vitamin C deficiency to gingivitis may be postulated on the basis of a sub-clinical deficiency, periodontoclasia is a rather severe type of tissue destruction and should be attended by other evidences of a frank deficiency before it may be regarded as a disorder of scorbutic origin (203)

*Caries* Claims that caries may be controlled or arrested by vitamin C therapy have not been substantiated As far as can be determined, there is no relationship between vitamin C deficiency and caries (272), nor is vitamin C therapy

effective in arresting caries (101) Westin (272) found no case in either guinea pigs, monkeys or man, in which scurvy had been a determining factor in the development of caries Grandison, Stott and Cruickshank (101) found no significant effect on dental caries following a daily administration of 200 mgm of synthetic vitamin C to 20 children for two years

*Vitamin C in extraction wound healing* Campbell and Cook (46) observed in eight cases that vitamin C given before, during or immediately after tooth extraction dramatically accelerated the healing of the extraction wounds In addition, they observed less postoperative pain and bleeding

**VITAMIN D DEFICIENCY** Vitamin D is concerned primarily with calcium metabolism and the calcification of the hard tissues Deficiencies in vitamin D are therefore reflected as deficient calcifications of the hard tissues—growing enamel, dentin and alveolar bone The changes in the rat following a diet deficient in vitamin D (but high in calcium and low in phosphorus) were recently studied in detail by Weinmann and Schour (262-266)

*Growing dentin* The earliest effect of an acute deficiency in vitamin D is the appearance in the dentin of a line of disturbed calcification (calcio-traumatic line) (262)

The response of the growing dentin to even a slight deficiency in vitamin D is so delicate that it can be used as an indicator of the adequacy of the vitamin D in the basal diet (218) Calcification of the dentin occurs by the coalescence of individual small calcospherites which begins at the predentin-dentin border In even mild deficiencies, the coalescence of the calcospherites is incomplete so that instead of a homogeneously calcified dentin, interglobular dentin results The dentin remains acidophilic and is dotted with the basophilic calcospherites instead of being homogeneously basophilic The normally sharp demarcation between the predentin and calcified dentin therefore also disappears

In more severe chronic deficiencies of vitamin D, the predentin does not become calcified at all, so that as the dentin matrix continues to be deposited, the uncalcified predentin border becomes wider and wider until in severe deficiencies it may attain a width of 90 microns instead of the normal 10 to 20 microns (142) (262) In very severe, prolonged deficiencies, pulpal inclusions may be found in the newly formed dentin (123) (11) (262) The rate of dentin formation is retarded (262)

*Pulp* Atrophy of the odontoblasts has been reported by Becks and Ryder (11), but has not been confirmed Blackberg and Berke (24) (25) observed inflammatory changes in the pulp Secondary dentin is also retarded in the rate of formation and disturbed in calcification (187) (190)

The effects of a vitamin D deficiency are markedly aggravated by (a) a deficiency in calcium and/or phosphorus (127) (168) (11), (b) a disturbance in Ca/P ration (127) (187) and (c) a deficiency in vitamin A (187) (125) In each instance the primary effect on the hypocalcification of the dentin is exaggerated

*Growing enamel* Enamel formation and calcification are apparently unaffected by vitamin D deficiencies The enamel organ shows no changes during enamel formation However, when the enamel organ reaches the stage when it is ready to atrophy, cystic degenerations may occur (262) This effect

takes place only after the enamel is completely formed and calcified (262) Apparently, enamel hypoplasia results only when the vitamin D deficiency is aggravated by a direct or indirect deficiency in calcium (187) or by parathyroidectomy (167) Becks and Ryder (11) did find enamel hypoplasia in rats subjected to vitamin D deficiency but it is likely that this deficiency was aggravated in their animals by some unknown factor (95)

*Alveolar bone* The newly deposited layer of bone is unable to calcify in vitamin D deficient animals, and so remains acidophilic and the normally thin osteoid border becomes very wide (11) (263) This uncalcified osteoid tissue also resists resorption (264) Weinmann and Schour (263) explain the increased thickness of the rachitic bone on the basis of a generalized lack of resorption and do not accept the explanation suggested in the term "compensatory hyperplasia"

*Periodontal membrane* The periodontal space is narrowed and compressed by the continued growth of the osteoid border of the alveolar bone and its lack of resorption, and eventually undergoes hyaline degeneration In the bifurcation of the rat molar, the periodontal membrane is crushed out of existence by the osteoid bone so that the latter is often in contact with the cementum (11) (263)

*Cementum* The formation of cementum matrix is normal but its calcification is defective so that the uncalcified precementum border becomes very wide (73)

*Eruption* Eruption of the teeth is retarded (263) (109)

*Changes in animals other than the rat* Changes essentially similar to those observed in rats have also been found in the growing dentin of dogs (187) (191) (24), and guinea pigs (116) In puppies fed a diet deficient in vitamin D and rich in cereals, the secondary dentin normally formed in response to caries or attrition is either absent or reduced in amount and imperfectly calcified (187) Becks and Weber (12) found osteoporosis and loss of supporting alveolar bone in dogs placed on a diet deficient in vitamin D In dogs placed on a diet low in vitamin D and aggravated by low calcium and high phosphorus, they observed a replacement of bone by fibro-osteoid tissue and an obliteration of the lamina dura and periodontal membrane

*Replacement Therapy in Rickets Vitamin D* Irving (123) and Weinmann and Schour (265) treated vitamin D deficient rats with vitamin D and observed an improvement in the calcification of the newly formed dentin The predentin becomes narrowed and sprinkled with calcifying globules and the healing occurs first in the oldest layers of the predentin, next to the calcified dentin (265)

*Phosphates* Administration of phosphate to vitamin D deficient rats results in a resumption of calcification in the oldest layers of the osteoid tissue at the end of the second day Osteoclasts reappear and resorption of the newly calcified tissues occurs (266) The dentin also shows improved calcification after phosphate therapy and a recovery to a normal rate of formation

*Starvation* Starvation of rachitic rats also produces a healing effect in the dentin (205) The possible effects of spontaneous fasting of animals during short-term experiments must therefore be kept in mind in evaluating disturbances in calcification

*Vitamin D Deficiency in Man Growing dentin* Typical rickets does not



occur except in the young growing organism with a definite need for calcium for the calcification of its growing hard tissues. The histologic changes in human teeth are similar to those seen in experimental animals and consist of deficient calcification of the growing dentin (interglobular dentin and a wide predentin) (98) (281) (139). A disturbance in the calcification of the dentin is not necessarily accompanied by a similar disturbance in the enamel (137). This has already been pointed out in experimental vitamin D deficiencies (262).

There is apparently a selective action of vitamin D in the calcification of the mesenchymal hard tissues (bone and dentin) since deficiencies in vitamin D do not usually affect the enamel.

*Enamel hypoplasia.* Vitamin D deficiency is generally believed to cause enamel hypoplasia in man. A review of the experimental and clinical evidence does not allow an uncritical acceptance of this view. In sixty cases of enamel hypoplasia, only ten patients had a history of rickets (216). Friel (89) reports an incidence of 28 per cent of enamel hypoplasia among Vienna children who suffered from a rachitogenic and otherwise deficient diet after the First World War. Infantile rickets does not always result in enamel hypoplasia and it is likely that, as is observed in experimental animals, enamel hypoplasia occurs when the vitamin D deficiency is aggravated by some other condition such as infantile tetany on a calcioprivic or parathyroprivic basis (235).

*Eruption.* The eruption of the deciduous and permanent teeth is retarded (89) (239).

*Malocclusion.* Malocclusion of rachitic origin has been reported. The imperfectly calcified alveolar bone is unable to withstand normal oral stress and therefore the teeth become maloccluded.

*Vitamin D deficiency and caries.* A number of clinical investigations have been conducted on the effect of vitamin D on caries, with conflicting results. Mellanby and her co-workers (190) (191) (192) have repeatedly suggested that optimal amounts of vitamin D will reduce the incidence of dental decay, although they were unable to produce dental caries in their experimental animals that were placed on a vitamin D deficient diet. McBeath and Verlm (176) reported a lower incidence of decay in children receiving a daily addition of 800 units of vitamin D in their diet. These findings, however, are not generally supported since many authors found no effect on caries when supplements of either calcium and/or vitamin D are added to the diet (170) (57). In spite of the increased intake of vitamin D in children during the last two decades, the incidence of decay has not shown any decrease (39). In addition, it must be emphasized that rickets is not necessarily accompanied by dental caries (89) (60). Youmans et al (284) observed no relation between extensive dental caries and calcium or vitamin D intake.

**HYPERVITAMINOSIS D.** A considerable literature has been developed on the deleterious dental effects of overdosage with vitamin D (105) (260) (18).

*Growing dentin.* Harris and Innes (105) report irregular calcification of the growing dentin in rats given excessive doses of vitamin D. A detailed histologic study correlated with blood calcium findings of the effects of single massive doses of vitamin D showed, in the dentin, an immediate *primary* reaction in the form of

a poorly calcified layer and a *secondary* reaction in the form of a well or excessively calcified layer (220) Schour and Ham (220) related the *primary* response to the rise in blood calcium and the *secondary* reaction to the *return* of the blood calcium to normal. Similar results were observed following the administration of parathyroid hormone (222) No effect was observed in the growing enamel

Massive doses of calciferol administered to parathyroidectomized rats resulted in an improved calcification of the dentin similar to the effect produced by the treatment with parathyroid extract (222) While the influence of vitamin D and the parathyroid gland upon the calcification of the teeth can hardly be questioned, the mechanism of their action and their possible interrelationship present unsolved problems for future research

**Periodontal structures** The alveolar bone becomes hypercalcified after massive doses of vitamin D The cementum is hyperplastic as well as hypercalcified so that in the molar teeth a partial obliteration of the periodontal membrane results (105) (260) (77)

In dogs, feeding of excessive amounts of vitamin D (with optimum amounts of vitamin A) results, according to Becks (18) in (1) generalized osteosclerosis of the jaws and alveolar bone, (2) severe pathologic calcification in the periodontal membrane and gingivae, (3) excessive formation of an atypical cementum leading to ankylosis between tooth and bone, (4) multiple pulp stones, and (5) extensive formation of calcium The addition of excessive amounts of vitamin A *lessened* the injurious effects

The rate of dentin and bone apposition, as measured by vital staining with Alizarine Red S was significantly increased following the daily administration of 78,000 U.S.P. units of vitamin D per kilogram to rats for a period of two weeks (285)

**VITAMIN E DEFICIENCY** Prolonged deficiency of vitamin E in the rat results in a loss of pigmentation of the enamel of the incisor (55) Davies and Moore (55) explain this on the basis of a secondary deficiency of vitamin A, although whitening of the teeth has been observed in rats suffering from vitamin E deficiency but who still had considerable vitamin A in the reserve Irving (124) found in addition a premature and abnormal degeneration of the enamel organ which he regarded as being possibly specific for vitamin E deficiency It must be pointed out that premature atrophy of the enamel organ is common in many chronic disturbances

**MINERAL DEFICIENCIES** **Total Salt Deficiency** Rats placed on a diet very low in all inorganic salts show severe disturbances in the calcification of the growing teeth and of the bone (7) (2)

The predentin becomes wider than normal and the amount of dentin formed becomes decreased until the incisors are mere shells and fracture readily In prolonged deficiencies, dentin formation is irregular and vascular (pulpal) inclusions occur In the molars, secondary dentin formation becomes irregular and pathologic cuspal fractures occur, followed in turn by carious lesions, pulpal necrosis and periapical abscesses Arnim et al (7) point out the fact that no enamel hypoplasias occurred in their animals

The alveolar bone is more severely affected than is the dentin The bony

trabeculae of the supporting spongiosa disappear and are replaced with fatty marrow tissue until only a thin shell of bone is left to support the teeth (7)

*Calcium and Phosphorus Deficiency* Rats placed on a diet deficient in calcium and phosphorus show disturbances in the calcification of the growing dentin and bone with but little effect upon the growing enamel (Gaunt and Irving, 1940)

*Growing dentin* Calcium and phosphorus deficiency results in a progressive increase in the width of the predentin, an irregular border between the predentin and dentin, interglobular dentin and, in severe chronic deficiencies, vascular (pulpal) inclusions (93) (38) (270) The width of the predentin, normally 10 to 20 microns, may reach 90 to 100 microns in direct ratio to the degree of deficiency and is a much more sensitive measure of the deficiency than the chemical (ash) analysis (92) (93) (143) or the blood calcium analysis (22)

*Growing enamel* Gaunt and Irving (95) found no changes in the enamel organ or in enamel formation or calcification However, Boyle and Wesson (38) employing a low-calcium diet with a P:Ca ratio of 16 to 1 observed premature atrophy of the enamel organ and, in severe chronic cases, disturbed calcification and hypoplasia Supplements of vitamin D prevented these effects (270) (38) Thus it is clear that in calcium and phosphorus deficiencies, as in vitamin D deficiency, the growing enamel is much more resistant to disturbances in calcification than are the growing dentin and bone

*Alveolar bone* The alveolar bone tends to show excessive resorptions (13) (12) This is in contrast to vitamin D deficiency in which resorptive activity is decreased because of the osteoid border (263) In calcium and phosphorus deficiencies, the effect upon the bone is seen before the effects can be noted in the dentin (7) (93)

*Alterations in Ca:P ratio* For the normal calcification of dentin and bone, the Ca:P ratio must be between 4:0 and 5:0 and the amount of these elements in the diet must be at least 0.3 per cent (95) If the calcium and phosphorus content of the diet is less than 0.3 per cent, rickets results The effect of a deficient intake is aggravated by changing the ratio of Ca:P Downs (67) observed disturbances in calcification when the Ca:P was either abnormally high or abnormally low Gaunt and Irving (95) showed that when the Ca:P ratio is lowered from 4:0 to 1:0 or 0.5, the effects upon the dentin are much more severe than upon the bone Vascular inclusions are common and the ash content of the bone is much less affected On the other hand, when the Ca:P ratio is high but deficient in amount the effects upon the bone are more severe than upon the growing dentin (95)

*Vitamin D therapy in calcium deficiency* Vitamin D therapy greatly lessens the effects of a diet deficient in calcium (38) (270) Boyle and Wesson (38) suggest, but do not prove, that the action of vitamin D in improving the calcification of tissues in animals on a low-calcium diet is due to an increased retention of calcium They also show that a high protein diet limits the effectiveness of the vitamin D

In rats on a calcium-phosphorus deficient diet, Irving (127) observed a resumption of calcification in the newly formed dentin 30 hours after the administration

of 18 41 u of vitamin D The amount of vitamin D necessary for the resumption of good calcification varied with the Ca: P ratio of the diet

*Multiple deficiencies in vitamin D and calcium* Vitamin D deficiency greatly aggravates the effects of a calcium-deficient diet (187) (127) Lund and Armstrong (168) fed rats a diet low in calcium and essentially vitamin D free They observed, on gross dissection, that the alveolar bone was soft and friable and the molars were loose The incisors were well developed Becks and Weber (12) observed marked resorptions and osteoporosis in the jaws of pups fed a diet deficient in vitamin D, low in calcium but high in phosphorus The supporting bone was entirely replaced by fatty marrow Toverud and Toverud (252) fed pups a diet deficient in calcium, phosphorus and vitamins A and D and found that the age of the animal and the duration of the experiment influence the severity of the results The disturbances in the calcification of the teeth were increasingly severe if the experimental diet began at the end of lactation, at birth or during gestation

Parathyroidectomy also aggravates the effects of a diet low in calcium (22)

*Difference in effect of calcium deficiency on dentin and bone* The effect of a calcium-deficient diet is much less severe in the growing dentin than in the bone (93) Dentin has a distinct priority for the available calcium since in mild deficiencies, dentin calcification may be normal while bone calcification is deficient (142) Erdheim (73) points out that certain tissues are less susceptible to disturbances in calcium metabolism than are others His calcioprotective law has been proved many times (7) (92) (49) (217) In addition, while bone may show marked resorptions and replacement by fatty marrow, the dentin does not Dentin is not subject to calcium withdrawal by resorption, as is the bone (226) In fact, the calcium and phosphorus made available by the bone are avidly taken up by the calcifying enamel and dentin (227)

*Adult teeth.* Efforts to alter the calcium content of the adult dentin in dogs by large doses of vitamin D and calcium over periods up to 135 days have been unsuccessful in spite of the fact that the calcium content of the blood was almost double the normal (78)

The molars of rats placed on a diet deficient in calcium show that the pre-experimental dentin is unaffected, but the secondary dentin, forming during the experimental period, is poorly formed and calcified (38)

*Calcium deficiency in man* Calcium is an important dietary consideration and deficiencies in calcium intake are very common in man (92) (93) (284) The changes in the growing dentin and bone are similar to those observed in experimental animals (175) (81) The fully formed enamel and dentin are not affected by calcium deficiencies or by any calcioprivic state (225)

Roentgenograms of the growing epiphyses readily reveal deficiencies in calcium and vitamin D in the growing child. This method of assessment is not applicable in the adult.

Since, however, the alveolar bone continues to grow throughout life and is composed, like the trabeculae under the epiphyseal plate, of spongy bone, dietary deficiencies related to mineral metabolism affect this bone quickly On the basis

of work now being conducted, the authors feel that intra-oral dental roentgenograms of the alveolar bone may show the effects of calcium deficiencies in the adult

*Calcium and caries* There is no established relationship between calcium therapy or calcium intake and caries (63) (170) Day (60) found that rachitic children and women suffering from osteomalacia in Northern India were remarkably free from caries Malan and Ockerse (170) studied a group of children who were fed daily supplements of calcium and phosphorus (0.5 gram each) and found no significant effect on the incidence of caries Rats on low-calcium diets do not show any increase in caries (38) Day and Sedwick (57) found that supplements of vitamin D had no significant effect on the incidence of caries Biochemical studies have shown that caries is not dependent upon the calcium content of the enamel or dentin (4)

*Iron deficiency* The oral manifestations of iron deficiency have been given much less consideration than those of calcium deficiency and merit investigation

*Magnesium deficiency* Rats placed on a diet deficient in magnesium show characteristic disturbances in both the calcification and the formation of the incisors Radiographic findings consist of a widening of the periodontal membrane, an indistinct lamina dura and a disturbed contour of the enamel surface (90) Eruption is retarded to one-third the normal rate (91) (68) Chemical studies show no change in the absolute amount of magnesium in the incisors, in contrast to the long bones which show a decrease (68)

*Calcification of dentin* Disturbances in the calcification of the dentin are characterized by a prominent stratification which is unlike that seen in any other known experimental condition The striations are sharply delineated and are repeated at intervals of 32 to 48 microns (121) It has been assumed that these striations are associated with the intermittent interference with calcification and the convulsive attacks of magnesium deficiency (91) However, Watchorn and McCance (258) also found these striations in subacute magnesium deficiency in which no convulsions occurred

*Formation of dentin* The rate of dentin apposition is progressively decelerated, the enamel-covered dentin being retarded almost immediately and more severely than the cementum-covered portion

The widths of the labial and lingual dentin are approximately the same in the normal animal In magnesium deficiency, the labial width is one-third or one-half that of the lingual width The pulpal outline, therefore, is characteristically distorted This finding is in direct contrast to that in vitamin A deficiency in which the rate of formation of the enamel-covered dentin is accelerated and the cementum-covered dentin is decelerated

Temporary local cessation of dentin growth may occur The rate of formation is also decreased in the growing dentin of the molars The retardation of the rate of dentin apposition and tooth eruption is greater than that of body weight (90) (91)

*Pulp* The odontoblasts show an early and progressive atrophy They are

reduced in size and become enclosed and calcified within the dentin matrix (258) The pulp shows various types of calcification (16)

*Growing enamel.* The enamel epithelium undergoes early atrophy and degeneration and in advanced stages of the deficiency, the enamel is severely hypoplastic (15) Irving (121) describes calcareous granules which become embedded in the enamel matrix at the basal end

*Periodontal structures* Klein, Orent and McCollum (158) reported a gross proliferation of the periodontal structures in their rats The gingivae were hypertrophied and the periodontal membrane showed edematous changes in the intercellular structure and an increased width The growth of the alveolar bone is decelerated to one-half or less of the normal rate (91)

*Replacement therapy* does not change the structures already formed during the deficiency but leads to a recovery of the odontoblasts and to normal eruption

**FLUOROSIS** Although fluorosis does not constitute a dietary deficiency, the presence of fluorides in the drinking water (and to a much lesser extent, in foods) of many communities and its effects on tooth development call for its consideration in this review There is no evidence that fluorine is *essential* to the body economy or to tooth development In fact, fluorine is an enzymatic inhibitor and a protoplasmic poison Fluorides exert a harmful effect when ingested in *excess* amounts, in contrast to most nutritional factors which affect the teeth and periodontal structures when they are deficient in amount

Fluorosis has been the subject of comprehensive reviews (195) (206) (103)

*Growing enamel.* The developing enamel is the first structure to react to the ingestion or injection of fluorides The ameloblasts show a selective response in the form of abnormal globules within their cytoplasm as early as one hour following a single injection of 0.3 cc of a 2.5 per cent solution of NaF (219) This is followed by the formation of sharp rings of disturbed calcification in the calcifying enamel and dentin The disturbance in calcification is probably brought about by the deposition of calcium fluoride in the tissue instead of the normal calcium salts ( $\text{PO}_4$  or  $\text{CO}_3$ ) and possibly also by disturbances of the enzymatic phosphatase system concerned in calcification (65) Irving (126) suggests that fluorine acts primarily on the composition of the blood

The demarcation of the growing enamel and dentin by injections of sodium fluoride permits an exact quantitative measurement of the rate of growth of enamel and dentin in animals and man (218) (219) (223) (127)

Sodium fluoride fed to rats results in different degrees of disturbance in the calcification of the enamel, depending upon the dosage and duration of the experiment The effects range from mild disturbances such as the loss of pigmentation and defective calcification in the enamel to severe enamel hypoplasias (230)

In man, the ingestion of water containing more than 5 parts per million of fluorides during the period of enamel (crown) formation and calcification results in a characteristic mottled enamel in 90 per cent of children In 35 per cent the enamel may be subject to hypoplasia and is permeable to oral stains so that it

of work now being conducted, the authors feel that intra-oral dental roentgenograms of the alveolar bone may show the effects of calcium deficiencies in the adult

*Calcium and caries* There is no established relationship between calcium therapy or calcium intake and caries (63) (170) Day (60) found that rachitic children and women suffering from osteomalacia in Northern India were remarkably free from caries Malan and Ockerse (170) studied a group of children who were fed daily supplements of calcium and phosphorus (0.5 gram each) and found no significant effect on the incidence of caries Rats on low-calcium diets do not show any increase in caries (38) Day and Sedwick (57) found that supplements of vitamin D had no significant effect on the incidence of caries Biochemical studies have shown that caries is not dependent upon the calcium content of the enamel or dentin (4)

*Iron deficiency* The oral manifestations of iron deficiency have been given much less consideration than those of calcium deficiency and merit investigation

*Magnesium deficiency* Rats placed on a diet deficient in magnesium show characteristic disturbances in both the calcification and the formation of the incisors Radiographic findings consist of a widening of the periodontal membrane, an indistinct lamina dura and a disturbed contour of the enamel surface (90) Eruption is retarded to one-third the normal rate (91) (68) Chemical studies show no change in the absolute amount of magnesium in the incisors, in contrast to the long bones which show a decrease (68)

*Calcification of dentin* Disturbances in the calcification of the dentin are characterized by a prominent stratification which is unlike that seen in any other known experimental condition The striations are sharply delineated and are repeated at intervals of 32 to 48 microns (121) It has been assumed that these striations are associated with the intermittent interference with calcification and the convulsive attacks of magnesium deficiency (91) However, Watchorn and McCance (258) also found these striations in subacute magnesium deficiency in which no convulsions occurred

*Formation of dentin* The rate of dentin apposition is progressively decelerated, the enamel-covered dentin being retarded almost immediately and more severely than the cementum-covered portion

The widths of the labial and lingual dentin are approximately the same in the normal animal In magnesium deficiency, the labial width is one-third or one-half that of the lingual width The pulpal outline, therefore, is characteristically distorted This finding is in direct contrast to that in vitamin A deficiency in which the rate of formation of the enamel-covered dentin is accelerated and the cementum-covered dentin is decelerated

Temporary local cessation of dentin growth may occur The rate of formation is also decreased in the growing dentin of the molars The retardation of the rate of dentin apposition and tooth eruption is greater than that of body weight (90) (91)

*Pulp* The odontoblasts show an early and progressive atrophy They are

refined sugars in the form of sweetened beverages and candies is desirable from the standpoint of public health since they tend to displace other essential nutrients (23)

Most dental investigators agree that the ingestion of excessive amounts of fermentable carbohydrates accelerates the progress of dental decay (128) (60) (41) Reduction of carbohydrate intake, on the other hand, tends in most cases to slow down or even arrest the progress of decay (60) The *Lactobacillus acidophilus* count, which is a dependable indicator of caries activity, can be raised by increased ingestion of carbohydrates or lowered by a decreased intake of carbohydrates (20) (128)

The highly refined sugars are rapidly broken down by bacteria with the production of free acid metabolites (84) The ingestion of large amounts over long periods of time as is found in children who habitually suck on large quantities of hard candies or in pastry bakers results in a characteristic "sugar caries" (194) The latter tends to be of a characteristic cervical type and apparently begins through a decalcifying action upon the enamel (194)

Experimental studies suggest that an excessive intake of carbohydrates does not initiate caries but rather increases the rate of decay already present (149) (58) (168) (209) (212) (52)

*Acid Foods* Although acid is produced by bacterial action incident to carbohydrate degradation (84), free acid may also be ingested with foods The latter tends to decalcify the enamel surface rather than to produce typical caries as occurs in the carbohydrate degradation by bacteria The local decalcifying action depends upon the concentration and amount of exposure (271) (155) (178) However, the buffering action of the saliva is usually sufficient to protect the enamel (85) (3)

The presence of citric acid in acidified candies constitutes a distinct hazard to the enamel West and Judy (271) found that a 40 per cent solution of acidified candies in water had a pH of 2.5 to 2.8 and dissolved enamel in six hours The manner in which children suck upon these candies over long periods of time permits a high local concentration upon the tooth surface (271) (194)

The effect of the indiscriminate use of concentrated beverage flavors and beverages containing large amounts of citric and tartaric acids (178) as well as the excessive use of lemons and lemon juice should also be considered in respect to their decalcifying action upon the enamel (155)

*Acid-Base Character of the Diet. Alveolar bone* The excessive ingestion of foods with an acid reaction or acid ash results in a generalized acidosis and in the withdrawal of calcium salts (by resorption) from the spongy bone (134) The urine becomes acid in reaction The supporting spongiosa of the alveolar bone shows a marked resorption and osteoporosis so that the teeth become loose (134) (135) (9)

*Caries* The possible rôle of acidosis or alkalosis in the production or prevention of caries is not so clear Jones (138) associated an excessive intake of acid ash foods with the presence of rampant decay Kugelmass et al (165) observed an arrest in the progress of decay when children were placed on an alkalinizing diet



often appears brown. The ingestion of water containing about 2 p p m of fluorides results in mild mottling in approximately 50 per cent of the children. The ingestion of water containing 1 p p m of fluorides results in a very mild mottling of the enamel (chalky-white spots) in about 10 per cent of the exposed persons (61) (62).

Biochemical analysis shows that the fluorides ingested during the period of development is stored in the hard tissues throughout life (6).

*Growing dentin.* The growing and calcifying dentin responds much less to fluorides than does the developing enamel. The calcification of the dentin becomes interglobular and stratified only after relatively large doses. Disturbances in the formation of the dentin matrix (vascular and pulpal inclusions) occur only in prolonged experiments and are probably the result of cumulative effects of fluorides (218) (230). Calcium has a protective action against fluorine intoxication and variations in the calcium and phosphorus content of the diet may modify the degree of response of the dentin (126) but fail to nullify the effect of the fluorine (237). Fluorine when added to the diet is most toxic in water, less in milk and least in mixed foods (111).

*Periodontal structures.* The periodontal structures show the least disturbance. In dogs, prolonged feeding of fluorides produces changes that can be recognized radiographically and that consist of an irregular and indistinct outline of the periodontal membrane and the alveolar bone trabeculae (206).

*Adult teeth.* Completely formed and calcified enamel and dentin are not affected by fluorides. An individual who has lived many years in an endemic area which contains sufficient fluorine in its water supply to produce mottling of the teeth in the children (usually more than 1 p p m) shows no effects in his teeth provided he settled there after tooth development had been completed (181).

*Fluorides and Caries.* Recent experimental, biochemical, clinical and bacteriologic investigations show that mottled enamel is less liable to dental caries (64) (5). Fluorides added to the diet inhibit the rate of experimental caries progress in the rat (193) (75). However, doses up to 10 p p m. must be used to be effective (177). Miller (193) explains the action of fluorides as an inhibition of the phosphorylation mechanism in the carbohydrate breakdown by the bacteria. Another possible explanation is that an optimal amount of fluorine in the enamel makes it more resistant to acid dissolution (5).

It has been estimated that in man the daily ingestion of 0.5 to 1.0 mgm of fluorides during the first eight years of life would reduce caries about 50 per cent (179) (62). This fact has led to many investigations attempting to utilize fluorides to prevent or lower the incidence of dental decay by topical application (161) or by adding fluorides to the communal water supply in minute quantities (1 p p m or less) (64). The dangers of chronic fluorosis call for caution and further investigation before current proposals to add fluorine to water supplies are given general acceptance (64).

**CARBOHYDRATES.** Carbohydrates are the primary source of energy for the body. However, the American people tend to consume excessive amounts of this nutritional element. Restriction in the excessive consumption of highly

reduced (160) Howitt, Fleming and Simonton (119) and Haydak et al (100) showed that a non-detergent or soft diet results in the rapid accumulation of epithelial and bacterial debris upon the surfaces of the teeth. Although the bacterial count is markedly increased by this type of diet, the actual effect on caries progress was not measured.

*Attrition* Klatzky and Klatell (156) and Day (60) point out that in primitive peoples, on a diet which caused marked attrition of the teeth, the incidence of caries is much less than in civilized peoples on a much softer diet which requires less masticatory function.

*Protective action of lipoids* Rosebury and Karshan (208) observed that while experimental caries in the rat was not prevented or cured by additions of vitamin D to the experimental diet, the rate of progress was retarded. They felt, however, that part of the protection lay in the vitamin free corn oil. In 1939 (210) they showed that vitamin-free corn oil, Wesson oil, Crisco and lard were effective in reducing the caries index. Box (29) and E V McCollum et al (180) have extended these observations to man. They believe that the lipoids in the diet may form a protective mechanical coating upon the teeth and thus prevent access to bacteria or their acids.

Thus it may be concluded that the physical character of the diet is an important consideration in the problem of dental caries.

*Physical Character of Food and Periodontal Disease* Hard foods which require thorough mastication are not only detergent in action but also stimulate the periodontal structures and promote the blood supply (45) and hornification of the gingivae (197). Thorough chewing leads to intermittent stresses which are transmitted to the periodontal structure and become transformed into intermittent tension forces upon the alveolar bone. The latter responds by reorganization and apposition. Soft and adhesive foods, on the other hand, lead to local gingival inflammation (197) (45), and may serve as a nidus for calculus formation. Haydak et al (106) fed a diet consisting exclusively of cow's milk and honey to five adult men. A heavy white cheesy film covered the teeth and gums. The gums were hyperemic and red. They explained these findings on the basis of a lack of stimulation and mechanical cleansing normally derived from the mastication of solid food. Mead (182) found that the daily addition of one and one-half grapefruit to a regular ration resulted in improvement of gingival health. It is quite possible that this result is due to the local cleansing effect of the fruit in addition to the possible benefits of increased vitamin C intake. The enamel showed no decalcification or other deleterious effects.

The evidence seems to be conclusive that foods when in a physical state which requires thorough mastication tend to promote oral hygiene and the health of the periodontal structures.

Sharp, splintery foods may be conducive to local injury when the food particles become embedded in the soft tissues and act as foreign bodies. An excessive diet of oats in rabbits, guinea-pigs, rats and mice produces widespread gingival necrosis and destruction of the supporting dental apparatus (259) (99) (274).

DIET AND DENTAL DISEASE. Any analysis of the influence of the various

nutritional factors on the diseases of the teeth must take into consideration the difference between the growing and the adult enamel and dentin. Hypocalcification (deficient calcification) and hypoplasia (deficient formation) of the enamel and dentin are of developmental origin and can therefore result only from nutritional disturbances occurring during tooth development. Dental caries, on the other hand, is an acquired post-developmental disease beginning upon the surface of the enamel and is therefore not influenced directly by nutrition but rather through factors present locally within the oral cavity. The periodontal diseases hold an intermediate position and can be influenced by both systemic and local factors.

*Dental Caries* Dental decay is not a developmental defect but an acquired, post-eruptive disease which attacks only the completed intra-oral portion of the tooth. The progress of dental caries is not influenced by the mineral content of the teeth except for fluorine (4). Many careful clinical investigations disprove the view that caries can be produced or prevented by altering the intake of vitamins or minerals (57) (60). Disturbances in calcification (as mottled enamel or rickets) or in formation (as enamel hypoplasia) also do not play a significant rôle in the etiology of dental caries but at best only influence the rate of its progress.

While a balanced, adequate diet is always indicated, it is in itself no guarantee against the ravages of tooth decay (207). Caries frequently occurs in individuals who enjoy optimal nutritional intake and can be produced experimentally in spite of a properly balanced diet (51) (207). On the other hand, caries has frequently been absent in individuals who suffered from nutritional deficiencies (159) (60). The low incidence of caries among native populations in different parts of the world has not been the result of any one dietary regimen (234).

In spite of these limitations in the effectiveness of a balanced diet in combatting dental caries, there have been indications on the basis of clinical (117) (118) (30) and experimental (207) data that an adequate diet may have some influence in retarding the rate of caries progress. The mechanism of such partial protection against caries is not known. It is probably related to a modification of the saliva and the oral milieu—a factor which appears to be all-important in the etiology and progress of dental caries (29).

The local action of the food upon the tooth surface may play an essential rôle in the initiation and progress of dental caries since food stagnation and decomposition on the surface of the enamel favor bacterial activity and acid formation, especially when the fermentable carbohydrates serve as the substrate (85). Dental caries is a problem of food decomposition in the oral cavity rather than of food absorption in the gastro-intestinal tract. The physical nature of the diet may thus be more important in preventing and controlling caries than the chemical content (60).

*Present status of caries research* The problem of caries is still unsolved and highly controversial (97). Experimental studies in animals have been complicated by the anatomical differences in the teeth of different species. Dog experiments have failed to produce caries (187). Continuously growing teeth in rodents do not decay since their rapid eruption and wear do not permit local food

stagnation and impaction. However, caries may occur in continuously growing teeth when retarded eruption and imperfect formation and shape permit food decomposition on the non-cleansing, imperfectly abrading surfaces (274) (77)

Clinical studies of caries for the most part have given inconclusive results because of inadequate and subjective methods of assessing caries activity and because of insufficient and inadequate controls (52)

*Hypocalcification and Hypoplasia* These clinical conditions are of developmental origin. The growing enamel and dentin are selective in their response to various vitamin and mineral deficiencies. Mild fluorosis will readily produce hypocalcification of the enamel without a corresponding response in the dentin. On the other hand, subclinical deficiencies in vitamin D or calcium will result in imperfectly calcified dentin without affecting the calcification of the enamel. These hypocalcifications are not uncommon. Enamel hypoplasias, however, result from permanent cell injuries and are rarely caused by a nutritional deficiency alone. For example, enamel hypoplasia cannot be predicted merely on the basis of an early history of rickets, but may be expected when there is a concomitant parathyroprivic tetany.

Once produced, hypocalcifications and hypoplasias are permanent and cannot be corrected by any dietary regimen. It must also be pointed out that affected teeth are not more prone to decay than are sound teeth.

The view that the depth of occlusal pits and fissures (which factor plays an important rôle in caries susceptibility) can be controlled by diet is erroneous. These structural defects are genetically determined and are the result of the fusion of the appositional growth centers established during tooth development at the relatively early stage of morphodifferentiation (227)

*Periodontal Diseases* The nutritional status of the patient must be considered in both the diagnosis and treatment of the periodontal diseases.

The periodontal tissues (gingivae, periodontal membrane, alveolar bone and cementum) are of continuous growth and sensitive to both systemic and local factors. Nutritional disturbances can affect their resistance and structure, as in the case of scurvy and pellagra. It must be pointed out, however, that the majority of cases are of local origin and their treatment consists of oral hygiene, local prophylaxis and the elimination of local irritants. When local treatment is ineffective, systemic factors must be considered. In apparently otherwise healthy patients who reveal no systemic disturbances, the possibility of a subclinical nutritional deficiency must be investigated and where indicated therapeutically treated.

#### SUMMARY AND CONCLUSIONS

The oral structures show early and characteristic changes in response to nutritional disturbances. Nutritional studies, therefore, should include gross and histologic examination of the following structures:

- 1 The enamel and dentin
- 2 The periodontal structures (gingivae, cementum, periodontal membrane and alveolar bone)

3 The tongue

4 The lips

5 The oral mucosa

The type of response varies with the particular dental or oral structure\* and its stage of development, with the specific nutritional disturbance, and with the species studied

The growing and calcifying enamel and dentin serve as a kymographic biologic record of the nutritional status of the individual and are especially sensitive to calcium, vitamins A, C, and D and fluorine

The fully formed and calcified enamel and dentin are no longer influenced by calcium metabolism. They contain the nutritional record of the past. The completed enamel is exposed to the oral fluids and oral flora and is beyond the pale of systemic nutritional influences. It is essentially devoid of an internal environment and is influenced by the latter only indirectly and to the extent that the oral milieu may be modified through systemic changes in the saliva or in the oral epithelium

The soft oral structures—the oral mucosa, the tongue, the lips, the gingivae and the periodontal membrane—which grow throughout life reflect the nutritional status of the present in both the young and the old individual and are especially sensitive to vitamins B, A and C

The alveolar bone, which also grows throughout life, responds particularly to disturbances in mineral metabolism and offers the advantage of radiographic examination

Our knowledge of the effect of nutritional disturbances on the oral structures is derived from both experimental and clinical observations

Most of the evidence on the delicate response of tooth development to nutritional disturbances is derived from animal experimentation, especially from rodents. Their incisors are of continuous growth and serve as ideal test objects for the analysis of growth, calcification and eruption

Where clinical data are not available, it cannot be assumed that the oral structures in man are equally sensitive, either qualitatively or quantitatively. There are considerable anatomic and physiologic differences in the dental apparatus of the rat, guinea pig, dog and other species

The findings on the response of the soft oral tissues are derived largely from clinical observations in man. This is especially true of the effects of vitamin B deficiency. The experimental and microscopic oral changes in this deficiency await further investigation

Present evidence has not established a specific nutritional basis for caries or for periodontal disease. Dental disease may be caused or aggravated by nutritional deficiencies and in such cases cannot be treated successfully unless the nutritional condition is recognized and corrected

The physical character of the food, through its cleansing action on the enamel surface and its stimulating action on the gingivae, is a significant dietary factor in caries and periodontal disease

Caries is largely a problem of food decomposition in the oral cavity rather than of gastro-intestinal absorption

The effects of proteins, fats and carbohydrates upon the teeth and surrounding tissues merit further investigation

Routine examination of the oral structures offers an accurate index to the state of nutrition of the individual

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# THE PHYSIOLOGICAL EFFECTS OF SUNLIGHT ON MAN

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The first problem confronting the writer of a review on the physiological effects of sunlight is to set the limits of his discussion, for sunlight exerts its influence indirectly upon so many aspects of life that one might embrace a vast field under this heading. When the topic is limited to the specific effects of sunlight impinging directly on the human organism, as it will be in the present instance, the field is greatly narrowed, but even then it is necessary to touch at least briefly on some of the indirect effects. For example, the heat load contributed by sunlight falling directly upon the human body is only one part of the total heat load to which the sun also contributes indirectly, and its physiological effects cannot be studied separately.

There exists a widespread belief in the beneficial effects of sunlight, which finds expression in the current fad of sun bathing. This belief stems in part from false analogies that have been drawn. For example, the idea that since sunlight is beneficial and necessary to plants it must be beneficial and necessary to man, which—however absurd—is found frequently in scientific literature, sometimes directly stated, sometimes implied. The detrimental effects of sunlight have received much less consideration and publicity, yet when one sets about the preparation of a critical review he is struck by the fact that the balance is weighted heavily on the side of effects which appear injurious rather than beneficial. Perhaps if some of the many claims were supported by better experimental evidence this would not be true, but if this review were restricted to a consideration of proven beneficial effects it would find a paucity of material. If, then, these pages seem to be more concerned with pathological processes than the reader may have expected, it is hoped they will not encourage him to adopt a troglodytic existence. Too much has already been written about the dire effects of tropical sunlight, and it would be unwise to revive the wearing of red spine pads or other paraphernalia designed to combat dangers that do not exist. It is hoped, however, that he may be led to recall Phaethon's misfortune at least as often as the triumphs of Mithra.

## PHYSICAL ASPECTS

Any review of this subject must presuppose knowledge of the physical factors involved, but the specific type of information required is not always readily available. Hence a brief consideration of certain aspects of the physics of sunlight, and of other essential matters precedes the discussion of physiological effects.

*The spectrum of sunlight* The maximum intensity of the solar spectrum just

<sup>1</sup> The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

outside the earth's atmosphere lies at about wavelength  $0.48\mu^2$ , which corresponds to the maximum for a black body at  $6000^\circ\text{K}$ , the temperature generally assigned to the outer surface of the sun. Small periodic changes in the intensity of sunlight reaching the earth's atmosphere may have important effects on climate, and hence on the behavior of mankind (e.g., 1, 144, 145) but so far as direct physiological effects are concerned, such fluctuations are unimportant, since variations in the intensity and spectral distribution of sunlight with season, latitude, and time of day, and with changes in the earth's atmosphere are so very much greater.

In passage through the atmosphere the spectrum of sunlight is modified because the atmosphere absorbs and also scatters some wavelengths to a greater extent than others. Ozone, which removes the shorter ultraviolet wavelengths, and water vapor which absorbs the longer infrared wavelengths are the principal absorbers, smoke, dust and the other gaseous constituents absorbing to a lesser extent. Scattering by gas molecules, by dust and smoke particles, and by water droplets affects the spectral distribution because short wavelengths are scattered more than long ones. Some of the scattered radiation reaches us as "sky-radiation" which, being relatively rich in the short ultraviolet wavelengths, may be a potent source of sunburn. The factors that determine the spectrum and intensity of sunlight at the earth's surface have recently been analyzed by Moon (202), who presents an approximate set of values based on the measurements of various investigators, which is useful in estimating the composition and intensity of sunlight under various conditions, the curves shown in figure 1 were drawn from his data. For values at the short wavelength end of the solar spectrum, particularly interesting physiologically, recent calculations by O'Brien (207) should be consulted.

Transmission of light by the atmosphere obeys the following equation very closely for any given wavelength,  $\lambda$

$$I_\lambda = I_{0\lambda} e^{-m_\lambda \sec z} \quad (1)$$

wherein  $I_\lambda$  is the intensity of radiation of wavelength  $\lambda$  arriving at the surface of the earth,  $I_{0\lambda}$  is the intensity of this radiation arriving at the outer margin of the earth's atmosphere,  $m_\lambda$  is dependent only on  $\lambda$ , and  $z$  is the angle which the sun subtends with the zenith, called the zenith angle. The length of atmosphere through which the sun's rays must pass (air mass) is directly proportional to the secant of  $z$ . When the sun is at zenith,  $\sec z = 1$ , and the sun's rays pass through the shortest possible length of atmosphere, or air mass 1. When the sun is at  $60^\circ$ , i.e., four hours from the zenith,  $\sec z = 2$ , and the sun's rays must pass through twice as great a length of atmosphere, i.e., air mass 2. In figure 1, curves 0, 1, and 2 represent solar spectra for these three air masses respectively. The curve for air mass 0, i.e., outside the earth's atmosphere, is obtained by extrapolation from measurements made at different zenith angles.

<sup>2</sup> Because of the wide range of wavelengths discussed herein, it will be convenient to use the micron ( $\mu$ ) as the unit of wavelength rather than the more customary units, the millimicron ( $m\mu$ ) and the Angstrom unit ( $\text{\AA}$ ).  $1\mu = 10^3 m\mu = 10^4 \text{\AA}$

The coefficient  $m_\lambda$  varies with the wavelength according to the extent of absorption and scattering by the atmospheric constituents, but remains approximately constant for a given wavelength. It is obvious from equation (1) that the magnitude of  $m_\lambda$  determines the extent to which the intensity  $I_\lambda$  varies with zenith angle, and hence with latitude, season and time of day, for a wavelength for which  $m_\lambda$  is large, the variation with zenith angle is greater than for a wavelength for which  $m_\lambda$  is small. Thus, for example,  $m_\lambda$  is very large for the sunburn producing wavelengths in the neighborhood of  $0.3\mu$  due to the fact that

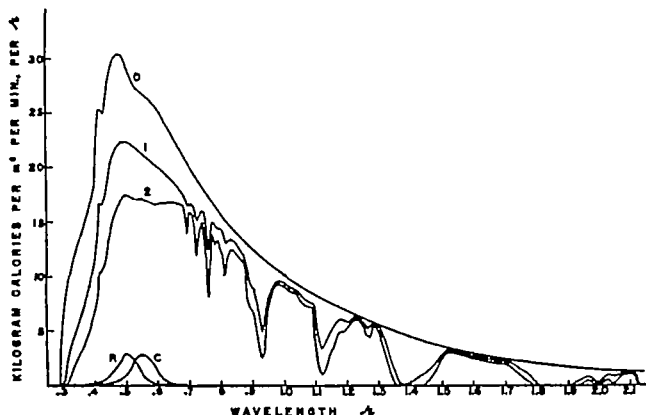


Fig. 1 Spectral distribution of sunlight 0 outside the atmosphere (air mass 0) 1, with the sun at zenith (air mass 1), 2 with the sun at  $60^\circ$  from zenith (air mass 2). Curves 1 and 2 are for 20 mm  $H_2O$  2.8 mm ozone and 300 dust particles per  $cm^3$ . From the data of Moon (202)

Curves R and C indicate respectively, the sensitivity of the human rods and cones the ordinate units for these curves are arbitrarily chosen

atmospheric ozone absorbs these wavelengths strongly, and hence the intensity of these wavelengths in sunlight varies widely with time of day. On the other hand,  $m_\lambda$  for the principal wavelengths perceived by the eye is relatively small since there is little absorption by the atmosphere in this spectral region. Thus, after five o'clock on a midsummer afternoon in low latitudes one is not likely to be sunburned, although the sun is still shining brightly insofar as the eye can determine. The deep indentations in the infrared region of curves 1 and 2 in figure 1, represent high values of  $m_\lambda$ , and correspond to absorption by water vapor. Obviously the infrared portion of sunlight varies more with zenith angle and with the quantity of water vapor than does the visible part.

The division of sunlight into ultraviolet, visible and infrared spectral regions is arbitrary, but useful. Light, or visible radiation is usually defined as that

radiation perceived by the human eye, and is often said to lie between  $0.4\mu$  and  $0.7\mu$ , the approximate limits of photopic vision, radiation of shorter wavelengths is referred to as ultraviolet, that of longer wavelengths as infrared. The arbitrariness of this classification is seen by reference to curves *C* and *R* in figure 1, which describe, respectively, the spectral sensitivity for photopic, and for scotopic vision, for any limits assigned to these curves cannot be exact because the sensitivity falls off very gradually as the curves approach zero. Actually the rods perceive radiation of wavelengths as short as  $0.3\mu$ , but the sensitivity is so slight that it cannot be shown on the scale of curve *R* (106).

It is desirable when discussing a specific photophysiological effect to specify the wavelengths that produce it, rather than merely to state that it is caused by, say, ultraviolet or visible radiation. The wavelengths that elicit a given photobiological effect are determined fundamentally by the spectral absorption of some photochemically active substance in the living system (p. 490), and such absorption may occur anywhere in the ultraviolet, visible, or very near infrared, depending upon the chemical constitution of the absorbing substance.

*Penetration into the human body* The depth of penetration of sunlight into the human body is an important factor to be taken into account in the interpretation of photophysiological effects. Measurements of transmission of light by the skin are subject to considerable uncertainty, and there are some conflicts in the literature, but there can be no doubt that the major portion of sunlight is absorbed before it has penetrated more than a few millimeters. Quite ridiculous hypotheses have been based on the belief that the sun's rays penetrate to the deeper tissues and as a result certain useless articles of clothing have sometimes been worn in the tropics<sup>3</sup>.

Skin is made up of a number of layers, all displaying reflection and specific absorption of different extent and character, and the depth of penetration of radiation depends upon a number of factors, the relative importance of which varies with the wavelength of the radiation. Consideration of the fate of beams of monochromatic radiation of a few selected wavelengths impinging upon the skin will serve to illustrate this.

A beam of radiation of wavelength  $0.28\mu$  is scattered in all directions before it has penetrated far into the corneum, due to the tiny flake-like elements composing this layer, which represent the remains of the dead epidermal cells. Reflection and refraction at the boundaries of optical discontinuity presented by these elements renders the corneum an effective diffusing medium. The protein of the corneum strongly absorbs this wavelength, with the result that only a small fraction of the incident beam is reflected back from the skin (176, 239). Absorption is greatly enhanced by diffusion since that radiation which is scattered laterally must pass through a longer path of absorbing material than that passing through normal to the surface. This is shown by the great increase in transmission that results when the corneum is infiltrated with a "clearing agent" of appropriate refractive index, which fills up the space between the elements and reduces the reflection and refraction at their boundaries (175), and by measure-

<sup>3</sup> e.g., orange-red underwear and helmet linings (219), and red spine pads (29, p. 280).

ment of the light diffused at wide angles (155) The attenuation of radiation by such a system may be approximately described by the following equation

$$I_{\lambda} = I_{0\lambda} e^{-k(\alpha_{\lambda}, s_{\lambda})l} \quad (2)$$

in which  $I_{0\lambda}$  is the intensity of the incident radiation of wavelength  $\lambda$ ,  $I_{\lambda}$  is the intensity of such radiation after passing through thickness  $l$  of the absorbing medium, and  $k(\alpha_{\lambda}, s_{\lambda})$  is the attenuation coefficient The absorption function  $\alpha_{\lambda}$ , and the scattering function  $s_{\lambda}$ , are mutually dependent, but vary to different extents with different characteristics of the attenuating medium, in this instance the various layers of the skin For example, after passing through the corneum to the living layers of the epidermis, the scattering of wavelength  $0.28\mu$  undoubtedly diminishes because the optical discontinuity at the cell boundaries is not as pronounced as at the boundaries of the elements of the corneum (54) Thus the equation must be applied with caution, but is useful in a general way, for example, it serves to remind one that the intensity falls off approximately exponentially as the depth of penetration increases, and that any estimation of maximum depth of penetration can mean only the depth at which some small fraction of the incident energy is still detectable In the case of wavelength  $0.28\mu$  the intensity is greatly reduced in passing through the corneum, and virtually none of it passes the epidermis to reach the most superficial blood vessels in the papillary layer of the corium Very little radiation of this wavelength is reflected back from the skin surface The functions  $\alpha_{\lambda}$  and  $s_{\lambda}$  also vary widely with wavelength

Radiation of wavelength  $0.5\mu$  also diffuses upon entering the corneum, but is relatively little absorbed Hence a large fraction is reflected back from the skin (48, 49, 84, 238), while another large fraction passes through the corneum and the living layers of the epidermis to the blood vessels in the corneum (9, 15, 212) Here the radiation is absorbed strongly by the hemoglobin of the blood The effectiveness of the corneum in scattering such radiation is demonstrated by the fact that the capillaries themselves may be rendered visible through a microscope if scattering is reduced in the corneum by a "clearing agent" such as immersion oil Since the absorption by the overlying layers is not great, some of the radiation diffusely reflected from the skin comes from the deeper layers in which the blood vessels are situated Thus, under ordinary illumination the color of the skin is influenced by the amount and degree of oxygenation of the blood in the superficial vessels, because some wavelengths are absorbed to a greater extent than others

At wavelength  $0.6\mu$  much the same situation exists except that there is little absorption by the hemoglobin, and considerable energy of this wavelength may reach the subcutaneous tissues (9, 15, 212)

Wavelength  $3.0\mu$  like wavelength  $0.28\mu$  is largely absorbed by the corneum Scattering is much less pronounced at this wavelength (117, 118, 213)

None of the numerous studies of skin transmission that have been made, presents a complete quantitative picture covering the ultraviolet, visible and infrared An attempt to fit together such a picture would entail a consideration

of the relative accuracy of the methods used by the various investigators, and it is doubtful that this would accomplish much beyond revealing a number of conflicts. A general summary of the clearly established facts may be helpful, however. Transmission is very low for wavelengths shorter than  $0.32\mu$ , a spectral region of particular interest biologically. Such radiation is largely absorbed in the epidermis, principally by proteins (175). Measurements have been made by a number of investigators (9, 155, 175, 212), which agree well for these and longer wavelengths in the ultraviolet. Anderson and Macht's (8) measurements have been frequently quoted as indicating much greater transmission, but Anderson and Fraser (7) using the same method later obtained values in line with those cited above. Bachem and Kuntz' (14) values seem somewhat high, whereas earlier estimates (122, 124) are too low because the methods used neglected much of the scattered radiation. Transmission increases progressively with wavelength through the near ultraviolet, and is high throughout the visible, considerable amounts of visible radiation reaching the corneum and even the subcutaneous tissue. Transmission remains high in the near infrared, but deep indentations appear in the curve at wavelengths of maximum absorption by water, and at the long wavelength limit of sunlight (about  $3.0\mu$ ), penetration is very slight (118). There is considerable disagreement as to maximum values for penetration, which various investigators place at from  $0.7\mu$  to  $1.5\mu$  (15, 53, 105, 138, 211, 212). The temperature of the tissues at some depth may be raised appreciably when radiation impinges on the skin surface (163), but this does not indicate actual penetration of the incident radiation, and Hardy and Muschenheim (117, 118) believe that the high transmission values obtained by some investigators result from the inclusion of radiation re-emitted from tissue heated in this way. The latter investigators found the maximum infrared transmission of human skin 2 mm thick to be about 6 per cent, whereas Cartwright's measurements (53) (also cited by Danforth, 69) indicated that over 20 per cent of radiation of wavelength  $1.15\mu$  passed through the human cheek, a thickness of 5 mm. Even if the highest values obtained could be accepted, it is not conceivable that an important fraction of sunlight reaches skin-tissues more than a few millimeters beneath the surface. The tremendous penetrations reported by Baldery and Ewald (18) are so out of line with any other findings that one is forced to conclude that faulty technique was employed. Those wavelengths that produce the most striking effect in human skin, sunburn, penetrate only a small fraction of a millimeter.

Little scattering takes place in the eye as compared to the skin, and consequently penetration is much deeper. The ocular media are very transparent to some wavelengths, but there is considerable selective absorption of others. There are no quantitative data for transmission in the ultraviolet, but it may be estimated from semi-quantitative measurements (81) that wavelengths shorter than  $0.32\mu$  are largely absorbed in the corneum and conjunctiva, although there is some penetration as far as the epithelium of the lens (251), this low transmission is no doubt due, in the main part, to absorption by proteins. Ludvigh and McCarthy's (179) measurements show only 9 per cent transmission at  $0.4\mu$ .

for the total eye media exclusive of the retina, but the transmission rises rapidly throughout the visible to reach a maximum at about  $0.8\mu$ . In the infrared we have the data of Hartridge and Hill (121) and of Roggenbau and Wetthauer (224) for the eye of the ox, and from these, estimates for the human eye may be made by correcting for the differences in thickness of the various layers. When this is done it is found that transmission to the retina falls to zero at about  $1.4\mu$ . This is approximately the same limit that would be set by a layer of water of the same thickness as the eye, and it is probable that this substance is the principal absorber of the infrared radiation of sunlight (121). Virtually all of the radiation that penetrates to the retina is absorbed by that structure, principally in the pigment epithelium.

TABLE 1  
*Reflection of total sunlight by human skin*

TYPE OF SKIN	REFLECTION	REFERENCE
	<i>per cent</i>	
Fine white	45	(183)
Average blond	43	(183)
Dark brunet	35	(183)
White	30	(209)
Dark partly negro	30	(183)
Hindu	22	(183)
Negro	16	(183)
Negro (I)	17	(209)
Negro (II)	19	(209)

*Reflection and emission.* A large fraction of total sunlight is reflected by human skin as is shown in table 1 which includes a number of measurements on different types of skin. It will be noted that the percentage reflected varies considerably for different complexions, but that less than half is reflected by the fairest skin. For detailed studies of spectral reflection the following references may be consulted 48, 49, 84, 117, 118, 176, 242, 238, 239.

There is virtually no reflection of wavelengths longer than those found in sunlight, the total radiation being absorbed very superficially. The human body, like any other body having a temperature in the neighborhood of  $37^{\circ}\text{C}$  emits a broad band of wavelengths with a maximum at about  $10\mu$ . In this spectral region human skin behaves virtually as a perfect radiator, or black body (116). This emitted radiation, which must be clearly distinguished from reflected sunlight, is often referred to as "black body radiation".

#### PHYSIOLOGICAL EFFECTS

Direct action of sunlight on the human body must be limited to superficial organs, the skin and the eye. In this review these direct, superficial effects will be considered first, after which phenomena resulting indirectly from them, which involve the deeper organs of the body, will be treated.



The direct effects themselves are of two kinds, a clear distinction between which should be made, (a) specific effects initiated by a photochemical reaction, and (b) non-specific or "radiant heat" effects, which merely result from a local rise in temperature. The former result from the activation of molecules by the capture of quanta of radiation, such capture constituting the primary act in a photochemical reaction. The type of chemical reaction which follows this primary act is determined by the kind of molecules present in the enviroing system as well as by the activated molecule itself (29). Such an effect is characterized by a specific *action spectrum*, that is, it is produced only by certain wavelengths that are specifically absorbed by the light absorber, i.e., the compound whose molecules are activated as the primary event in the underlying photochemical reaction. The action spectrum may follow the absorption spectrum so closely that the absorbing compound can be identified with considerable assurance, but the agreement is often masked by other factors (29, 36).

"Radiant heat" effects also result from the capture of quanta of radiant energy by molecules, but such capture is not followed by chemical reaction in this case. Instead, the energy of the absorbed quanta is distributed among the molecules of the system in such a way as to increase the average kinetic energy, and hence the temperature of the system. Whether a photochemical reaction occurs or not depends upon the type of molecule that captures the quantum, and also upon whether other appropriate molecules are present to participate in a photochemical reaction. If such conditions do not exist the absorbed energy will go toward heating the system, this is the fate of most of the energy of sunlight that enters the human body. Although the term is often employed, no specific "heat rays" exist, in the sense of the specific wavelengths that produce photochemical reactions in the eye or in the skin.

**EFFECTS ON THE SKIN Sunburn** Exposure of the skin to bright summer sunlight for half an hour or longer is followed by dilatation of the minute vessels of the exposed area, manifested grossly as erythema. The erythema is accompanied by swelling, often so slight as to be almost imperceptible. If the exposure is prolonged, marked edema, desquamation or blistering may follow, and there may be pain or itching. The erythema fades in the course of a few days, being gradually replaced by "suntan" due to rearrangement and increase of melanin in the epidermis. The suntan may persist for months or even years. In addition the suntan may darken by a process beginning almost immediately upon exposure, and ceasing with the exposure. All these events have a common origin—immediate or remote—in the action of ultraviolet radiation on the epidermis, and may be logically included under the term *sunburn*, even when the observed response is minimal and may not seem to merit classification as a "burn."

The writer's concept of sunburn postulates that direct injury to the cells of the epidermis by ultraviolet radiation is the crucial mechanism underlying the events enumerated above, and that as a result of such injury the cells elaborate (i.e., produce and release) various substances which bring about specific physiological responses. The long latent period between exposure and the physiological responses may be accounted for by assuming that the physiologically active

substances are elaborated by the injured cells at relatively slow rates. Certain hypotheses based on the direct photochemical formation of physiologically active substances find difficulty in explaining the long latent period and duration of the physiological responses (p. 497). A scheme designed to represent the various events of sunburn and the processes underlying them appears in figure 2. Some of the steps in this scheme are much better substantiated by experimental evidence than others, and the pages immediately following will be devoted to an evaluation of this evidence. Various aspects of sunburn will be discussed, for convenience, under separate headings; this treatment is not intended to imply that they are separable as regards basic underlying mechanism.

*The basic process.* Ultraviolet radiation produces injurious effects in living systems in general, indicating that this agent acts upon some component of all

SCHEME TO DESCRIBE THE PRINCIPAL EVENTS IN SUNBURN

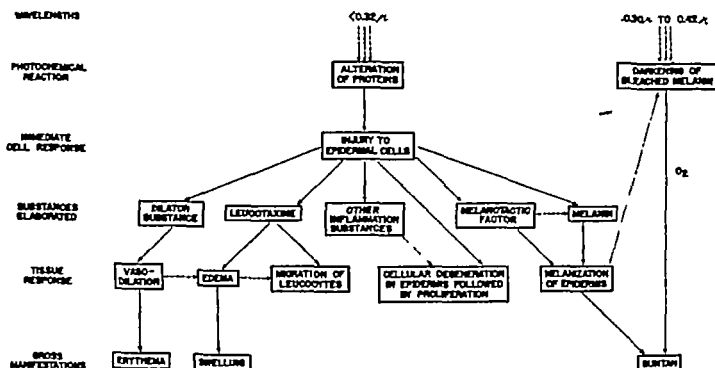


Fig. 2 A tentative scheme to describe the events of sunburn

cells—a concept strengthened by the fact that, with very few exceptions, such effects have their long wavelength limits at about  $0.32\mu$ , and have similar action spectra. Systems comprised of single cells such as bacteria, protozoa, the sperm and eggs of invertebrate animals, etc., lend themselves to the study of action spectra for injurious and lethal effects, many of which have been determined on such systems. All these action spectra show remarkable similarity to the absorption spectra of typical unconjugated protein or of nucleic acid (fig. 3). The universal importance of these two substances, their presence in quantity in all cells, and the fact that both are photolabile, leads to the conclusion that ultraviolet radiation exerts its injurious action by altering either or both (see 31 for discussion and references). It appears that proteins may be principally

concerned in some instances, nucleic acid in others, there being some evidence that in cells with considerable cytoplasm to absorb the ultraviolet radiation protein is the principal substance attacked

The first action spectrum for the erythema of human skin was obtained by Hausser and Vahle in 1922 (125)<sup>4</sup> This was revised by Hausser in 1928 (124), and determinations by Lukiesh, Holladay and Taylor (176), and by Coblentz, Stair and Hogue (63) appeared shortly after All these measurements are in essential agreement, and a standard curve for the erythema spectrum, by which

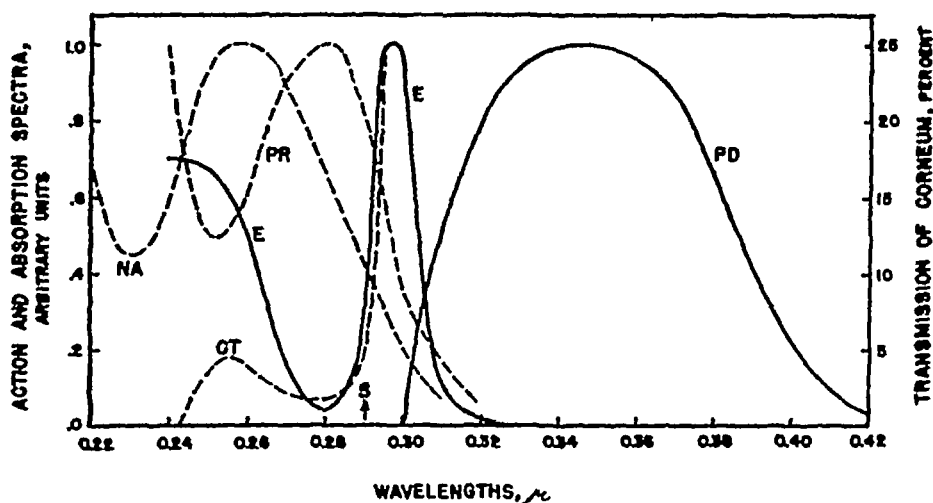


Fig 3 *E*, erythema spectrum, *NA*, absorption spectrum of a nucleic acid, *PR*, absorption spectrum of a protein, *CT*, transmission of human corneum, *PD*, action spectrum of pigment darkening reaction, *S*, lower wavelength limit of maximum sunlight at the earth's surface

Ordinates for the action, and absorption spectra, arbitrarily chosen to make the maxima correspond The relative threshold energies required for erythema, and for pigment darkening are, respectively, 1 and 600 for the wavelengths of maximum action (see 36)

name this action spectrum is generally known, has been formulated by Coblentz and Stair (61)

The erythema spectrum is represented in figure 3, where it may be compared with absorption spectra of a typical protein and of nucleic acid General

<sup>4</sup> The first clear recognition that sunburn is not caused by heat, but by an invisible portion of the sun's rays, seem to have come with the accidental finding that the same phenomenon results from exposure to the radiation emitted by electric arcs An account by Charcot (55) states that the physicists Foucault and Despretz had made this discovery sometime previous to 1858, and that Foucault correctly attributed the effects to ultraviolet radiation Only in 1889, however, was there clear-out experimental demonstration, when Widmark (255, 256) showed that the rays of the carbon arc passing through quartz produced sunburn, whereas those rays passing through glass did not Since glass cuts out much of the ultraviolet radiation which is passed by quartz, this experiment set the long wavelength limit for sunburn somewhere in the ultraviolet, and showed that visible radiation is incapable of eliciting the response Although this finding was soon confirmed, no more precise determination of the action spectrum was made until 1914, when Henri and Moycho (130) carried out such measurements for the erythema of rabbits' ears

similarity is apparent in that all three spectra have approximately the same long wavelength limit near  $0.32\mu$ , and that each has a discrete maximum, but the erythema maximum at  $0.297\mu$  does not agree with either the protein absorption maximum at  $0.28\mu$  or the nucleic acid absorption maximum at  $0.26\mu$ . Since the erythema spectrum, is measured at the skin's surface, however, it is not a correct index of the photochemical reaction underlying erythema, because this reaction takes place in the living layers of the epidermis, beneath the dead corneum which acts as a semi-opaque screen by absorbing the active wavelengths (pp 486-488). A transmission spectrum for corneum, presented in figure 3, shows that this layer has a minimum of absorption at about  $0.28\mu$  (indicating its essentially protein character), which corresponds to the deep minimum in the erythema spectrum at that wavelength. It was Hausser (124) who originally pointed out that this maximum of absorption by the corneum accounts for the minimum in the erythema spectrum.

Taking into account the absorption by the corneum, and making certain reasonable estimates, Mitchell (201) finds that the shape of the erythema spectrum can be explained on the assumption that protein in the malpighian layer of the epidermis is the light absorber for the photochemical reaction that underlies sunburn, his is the most complete analysis of the problem that has been presented. Hamperl, Henschke and Schultze (114), on the other hand, account for the erythema spectrum in terms of the absorption spectrum of nucleic acid, and suggest that this substance is the light absorber. They state that they have found maxima in the erythema spectrum at  $0.295\mu$  and  $0.28\mu$  with a minimum at  $0.275\mu$ , and for some skins with very thin corneum another maximum at  $0.23\mu$ , but unfortunately their analysis is described rather incompletely in a brief note, which makes its evaluation difficult. In support of their thesis, these investigators point out that the nuclei of the more superficial cells of the epidermis are the first to show degeneration, and suggest that this occurs because these cells receive more ultraviolet radiation than those more deeply placed, this may not be a cogent argument.

At this point there should be mentioned some uncertainties inherent in the erythema spectrum, which render the identification of the light absorber relatively inexact. The erythema spectrum is based essentially upon the minimal perceptible erythematous dose, i.e., the minimum amount of ultraviolet radiation which elicits just perceptible reddening of the skin. But the rate of development and fading of erythema is different for different individuals (Schall and Alus, 233), hence the minimum perceptible erythema depends upon the subject chosen as well as on the time of observation, and is not an exact index of the underlying photochemical process. Hausser (124) has shown, moreover, that the relative rate of development of erythema is different for different wavelengths. The expectation that action spectra and absorption spectra will agree is based on the assumption of the reciprocity law, *intensity  $\times$  time = a constant*, which does not hold for the erythema threshold at low intensities. The measurements of different investigators are in general agreement, but the amount of variation in detail (63) suggests individual variation which might indeed be expected to occur because of differences in transmission by the corneum (see also pp 486-488).

When the relative inadequacy of measurements of absorption by the layers of the epidermis is taken into consideration in addition to the sources of uncertainty already mentioned, it becomes obvious that any attempt to identify the light absorber for sunburn can be only tentative. No definite choice can be made between protein and nucleic acid on the basis of this evidence alone, and there is room for even further divergence of opinion in identifying the light absorber, other possibilities having been suggested in connection with hypotheses which will be discussed later in this review.

Relatively small amounts of ultraviolet radiation cause physical changes in proteins *in vitro* (10, 72), and it is not improbable that these provide the basis for injury to the cell. But nucleic acid is also altered physically by small doses of ultraviolet radiation (141), and this might also be the basis for cell injury. The erythema of sunburn is not inhibited by depriving the skin of  $O_2$  during the period of irradiation (44, 131), a fact that serves further to identify this process with the action of ultraviolet radiation on other organisms and biological systems, some of these systems are essentially protein and do not contain nucleic acid (29, pp 114-117).

Considered as a whole the evidence suggests that protein is the light absorber in the sunburn mechanism, and it is credited with that rôle in the scheme presented in figure 2. Whether protein or nucleic acid or both act as light absorbers makes no difference insofar as the subsequent steps in the scheme are concerned, once the postulate is accepted that ultraviolet radiation causes injury to epidermal cells.

Such injury is indicated by the histological changes accompanying sunburn, which have been the subject of a number of studies, among which those of Keller (151), Miescher (196), and Hamperl, Henschke and Schultze (115) may be cited. There are points of disagreement, due largely no doubt to differences in the dosage of ultraviolet radiation and in the time at which the biopsies were made, but on the whole the same general picture is presented by all these studies. In no case have histological changes been observed prior to the appearance of gross erythema, at which time enlargement and engorgement of the capillaries becomes marked, intracellular edema, and the migration of leucocytes into the epidermis and into the corium in the region of the capillaries appear about the same time or somewhat later. As early as 24 hours after the exposure degenerative changes in the prickle cells are detectable, these progress and may eventually involve all of this layer. The basal cells are less affected as a rule, but may also show degenerative changes, repair usually takes place through proliferation of cells of this layer. When the acute stage is passed all layers of the epidermis with the exception of the basal-cell layer, but including the corneum, are usually left thickened. Miescher found that with large doses, endothelial cells and fibroblasts of the corium, as well as epidermal elements, show degeneration. Changes involving melanin pigment occur, which will be discussed later.

*Erythema and inflammation.* The appearance of alterations in the vessels of the papillary layer before changes are observed in the epidermal cells, might seem to indicate that the primary action of the ultraviolet radiation is on the

former tissues. There is strong evidence to the contrary, however. The fraction of sunburn producing ultraviolet radiation that reaches the papillary layer is small for even the most penetrating wavelengths, and some wavelengths quite effective in producing erythema virtually do not get through the epidermis. The long latent period which usually intervenes between exposure and vaso dilatation, and the lack of degenerative changes in the papillary layer, except with severe dosages, support the concept that the site of injury is chiefly in the malpighian layer. Hence it is reasonable to assume that the vasodilatation results, in the main at least, from the elaboration of a dilator substance by the injured cells of the epidermis, which diffuses thence to the minute vessels of the papillary layer where it exerts its effect. This sequence of events is indicated in the above scheme. Increased concentration of dilator substance has actually been demonstrated in the skin (92, 150, 205), and in the blood of animals that have been exposed to ultraviolet radiation (157, 164).

The identification of the dilator substance released as a result of exposure to ultraviolet radiation has been the subject of considerable speculation. Lewis and Zotterman (169) suggested that it is histamine or a histamine-like "H" substance—a view that has been widely accepted. In recent years evidence has accumulated to indicate that histamine is not the dilator substance involved in various responses that resemble the histamine wheal much more closely than does the erythema of sunburn (3, 110, 189, 230), and the term "H" substance, as used to denote a substance pharmacologically similar to histamine, seems to have lost much of its usefulness and popularity. Specific differences between the effect of ultraviolet radiation on the skin and the histamine response have been shown (216), and Krogh (159) found it necessary to postulate at least two dilator substances to account for the behavior of the erythema of sunburn. Thus, although the elaboration of dilator substance seems to be the immediate cause of the erythema of sunburn, no definite statement can be made regarding the chemical nature of this substance.

Essentially the same tissue responses occur in sunburn that occur in inflammatory processes in general, and it seems not unlikely that the same underlying mechanisms operate in bringing about these responses. Menkin (190, 192, 193, 194) has recently isolated from inflammatory exudates several substances which act specifically to bring about certain of these tissue responses, and it seems probable that the complicated picture which inflammation presents may ultimately be explained in terms of such "inflammation-substances". This general thesis has been followed in introducing such substances into the scheme for sunburn outlined in figure 2. Menkin (189) has brought forth evidence that the increase in capillary permeability which occurs in inflammation is not due to histamine, as supposed by Lewis and others, but to a substance which he calls "leucotaxine". This substance also exerts a "chemotactic" effect, and by virtue of both these actions brings about the migration of the leucocytes from the capillaries into the surrounding tissues. In the scheme, leucotaxine has been included as a substance elaborated as the result of cell injury, and is credited with bringing about both the migration of leucocytes and tissue edema, the latter

being a direct result of increased capillary permeability. Indicated by a dotted line is the probable participation of vasodilatation as a lesser factor in the edema. A blanket caption has been used in the scheme to include other inflammation-substances. A substance, "necrosin", which Menkin (192) finds to cause the degeneration of cells, may be responsible in part for such changes in the epidermis and in the corium, but since degeneration of epidermal cells may also be expected to follow as a direct result of injury by ultraviolet radiation, both possibilities are indicated in the scheme. The possibility that inflammation substances exert systemic effects will be discussed later (pp 517-518). It should be emphasized that none of these mediating substances has been isolated from sunburned skin, with the possible exception of the dilator substance, and that the scheme has not been introduced with the expectation that all its steps will be demonstrated experimentally, but to give a better picture of the complex set of processes which constitutes sunburn.

A number of other changes, not indicated in the scheme, are associated with sunburned skin, the surface temperature is raised (163, 173), evaporation of water is affected (173, 186), there are changes in electrical potential (154, 248), there is an increase in permeability of lymphatics (143), hyperesthesia occurs (240), and there are changes in chemistry including enzyme activity (2, 149, 150, 259, 260). It is probable that all these changes are inter-related with the local inflammatory process, and need no special consideration. Changes in melanin pigment will be discussed below under a separate heading.

The various wavelengths of the sunburn producing radiation penetrate to different depths (note curve *CT* in fig 3), and it is possible that they exert somewhat different effects because they reach different layers of the skin. For example, measurements of the transmission of whole epidermis (155, 175) show that wavelengths shorter than  $0.28\mu$  do not penetrate below the epidermis, but wavelengths as long as  $0.31\mu$  may reach the corium, perhaps, in sufficient quantity to exert an effect at that level. Hausser (124) has shown quantitative differences in the effect of different wavelengths which suggest a relationship to depth of penetration. Those histological studies that have been made provide no answer to this question since all have employed spectrally inhomogeneous radiation, the effects of short and long wavelengths being thus included in the same picture. It is possible that the changes in the corium which Miescher (196) and Keller (151) describe are due to direct action of the longer wavelengths of the erythema spectrum which may penetrate to these layers, but on the other hand, they may be due to a cell-injury substance (192) elaborated in the more superficial cells. That still more deeply penetrating wavelengths, longer than those of the erythema spectrum, are not responsible for any of the histological changes described above, is shown by the studies of Hamperl, Henschke and Schultze (115). Although the evidence indicates that the major part of the vasodilatation—probably all that produced by the shorter wavelengths—is due to the action of a dilator substance elaborated in the epidermis, a direct effect of longer wavelengths of the erythema producing spectrum on the vessels of the papillae cannot be completely ruled out.

Several theories for the erythema mechanism have been proposed which postulate that a dilator substance is formed directly by the action of ultraviolet radiation on components of the cells Ellinger (90, 91, 92, 93, 94) and Frankenburg (102) suggested that the radiation acts directly upon histidine, presumably in polypeptide chains, with the result that histamine is formed There are several objections to this hypothesis While a dilator substance, presumably histamine (but see 249, and 29 p 182) is formed in vitro by the action of ultraviolet radiation on histidine, the wavelengths absorbed by histidine do not correspond with the erythema spectrum (45, 94) If histamine is formed by direct action of the ultraviolet radiation the long latent period before erythema appears can only be accounted for by a slow diffusion process (93, and see 29 p 182) and a molecule as small as histamine might be expected to diffuse much too rapidly Moreover, the high temperature coefficient of the latent period (see p 504) hardly seems characteristic of diffusion of such a small molecule The long duration of the erythema, which may be present for several days after exposure, would demand that histamine continue to be formed long after the initial photochemical reaction is completed Objections to the concept that histamine is the dilator substance in sunburn have been cited above

Mitchell's (201) analysis of the erythema spectrum, showing that protein may well be the light absorber, has already been discussed He suggested that the dilator substance is a breakdown product of protein, formed by the direct action of ultraviolet radiation on protein molecules, a concept more acceptable than the histamine hypothesis, since high molecular weight breakdown products of protein might diffuse more slowly than histamine, and the process might be expected to have a higher temperature coefficient The long duration of the erythema, however, indicates that the dilator substance continues to reach the vessels for several days after the exposure, and it seems unlikely that a simple diffusion process would continue for such a long period

Rothman and Rubin (229) suggest that para amino-benzoic acid is the light absorber, but have not made a thorough analysis of the agreement of the absorption spectrum of this substance with the erythema spectrum They found that when solutions of this compound which had been exposed to ultraviolet radiation were injected intradermally, erythema of the local area developed after an appropriate latent period These changes did not occur in the absence of molecular oxygen, whereas deprivation of  $O_2$  during the period of exposure to ultraviolet radiation does not inhibit the subsequent development of erythema (44, 131) Another objection pointed out by the proponents of this hypothesis themselves, is that the injection of the irradiated para aminobenzoic acid solution causes erythema but no subsequent pigmentation, whereas these two events are virtually inseparable sequelae to the exposure of normal skin to ultraviolet radiation

*Suntan* As the erythema fades it is replaced by suntan, the transition from the one state to the other being almost imperceptible Quantitative studies of the spectral distribution of the radiation reflected from tanned and from unexposed skin indicate that their difference in color is due principally to the amount and position of the melanin pigment in the former (84, 85) In normal untanned



skin the melanin pigment is located chiefly in the cells of the basal-cell layer. A few days after exposure to ultraviolet radiation—about the time suntan first makes its appearance—the pigment begins to migrate into the more superficial epidermal layers, eventually reaching the corneum. As a result the basal cells may appear after some days to be almost free of melanin. The movement of melanin toward the surface causes the skin to present a darker appearance to the eye, even though the total amount of melanin is not increased at this early stage of suntan. Elaboration of new melanin is a later event, which is accomplished chiefly in the region of the basal cell layer (115, 151, 180). According to some investigators (e.g., 20) special dendritic melanoblasts form the melanin which they transfer to the basal cells, but according to Bloch (25, 26) and Peck (214) the dendritic cells are epidermal elements which share with the basal cells the ability to form pigment.

The migration of melanin into the superficial layers of the epidermis in sunburn (*Pigmentverschiebung*) reported by Keller (151) has also been noted by Peck (214) in pigmentation brought about by Thorium X (*Pseudohyperpigmentation*), it is suggested in the earlier observations of Lutz (180). This event occurs too early to be ascribed to epidermal hyperplasia. To account for this movement of melanin a "melanotactic factor" has been introduced into the scheme of figure 2, but without intending to assign a mechanism. Elaboration of melanin is also indicated, to represent the subsequent activity of the melanoblasts. It seems possible that the latter process is stimulated by the former, and this is suggested by a dotted line in the scheme. Both processes are represented as contributing to *melanization* of the epidermis—a term which will be used herein to include both migration and formation of melanin—and as having their origin in the injury to epidermal cells by ultraviolet radiation.

Raper, who with his collaborators has contributed much to our knowledge of the chemistry of melanin gives a series of reactions for the formation of this compound from tyrosine (see fig. 4). The first step is the oxidation of tyrosine to 3,4-dihydroxyphenylalanine, commonly known as dopa. This reaction takes place relatively slowly, and requires the participation of a catalyst, the enzyme tyrosinase, which has been isolated from numerous plants and lower animals but has not been shown definitely to exist in mammalian skin. In 1937 Arnow (11) found that ultraviolet radiation can replace the enzyme in this first step of the reaction, to bring about the oxidation of tyrosine to dopa. The exposures to ultraviolet radiation which were required were, however, much greater than those that produce suntan in human skin, but Rothman (228) found that the reaction is accelerated by ferrous salts, so that the amount of ultraviolet radiant energy required is comparable to the amount needed to produce suntan.

The concept that the initiation of the first step in Raper's reaction scheme is accomplished in the skin by the action of ultraviolet radiation meets a number of objections when the attempt is made to relate it to the events of suntan. Melanin appears as a sequel to the action of diverse agents injurious to the skin, for example, heat, friction, photodynamic action, alpha, beta and gamma

radiation, and wounds, as well as sunburn, and it is not likely that the same set of chemical reactions would be brought about directly by all these diverse agents. According to the hypothesis suggested by Arnow (11) and Rothman (228), the long latent period between exposure to ultraviolet radiation and the appearance

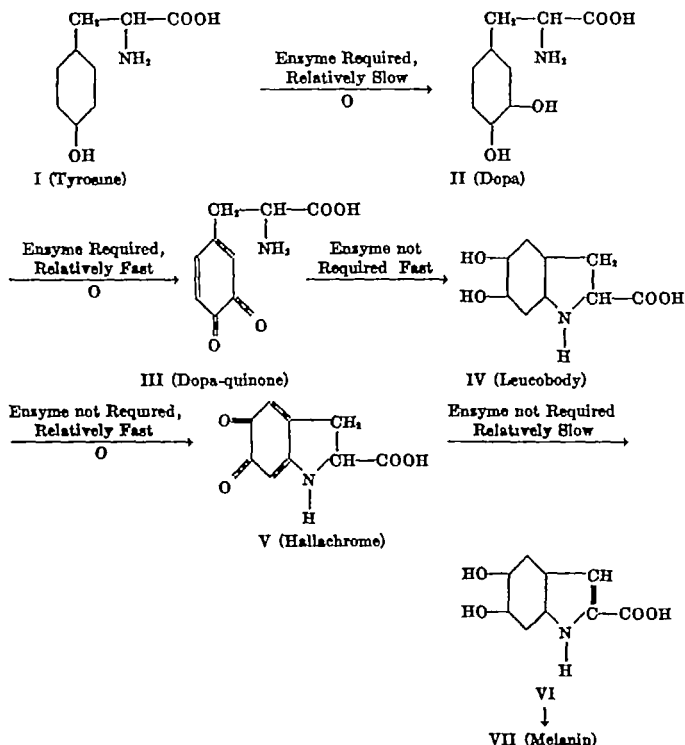


Fig 4 Reaction scheme for the *in vitro* formation of melanin from tyrosine (From Evans and Raper 100.)

of suntan—a matter of days—would have to be occupied by the first reaction (I to II) in Raper's scheme, and it is doubtful that it would fill the time between exposure and the elaboration of melanin in the melanoblasts, which does not begin until well after suntan has made its appearance due to migration of the preformed pigment. The melanoblasts are exposed to relatively little

sunburn-producing ultraviolet radiation because of their deep position in the epidermis. The *in vitro* transformation of tyrosin to dopa proceeds with the uptake of molecular oxygen, whereas, in contrast, the subsequent appearance of suntan is not inhibited by cutting off the oxygen supply during the exposure (44, 131), and thus if the oxidation of tyrosine to dopa during exposure to ultraviolet radiation takes place *in vivo* the oxygen must be obtained from some source other than  $O_2$ .

Bloch (25, 26) assigned a somewhat different, and in most ways more acceptable, mechanism to the elaboration of cutaneous pigment, which does not depend upon specific participation of ultraviolet radiation. His concept has been further developed by Lutz (180) and by Peck (214). When histological sections are treated with levorotatory  $\beta$ -3-4-dioxyphenylalanine (l-dopa), melanin is formed in the melanoblasts, presumably due to the presence of a substrate specific enzyme, dopa-oxidase, in these cells. Other closely related substrates, including the optical isomer, do not produce this "dopa reaction" (215). Epidermal injury by ultraviolet or other agents causes the melanoblasts to exhibit the dopa reaction, at first to a diminished, but later to an enhanced degree (180, 214). Presumably, levorotatory dopa, brought to these cells by way of the blood stream is converted, to melanin by the action of this oxidase. Peck (214) suggests that this specific reaction is favored by conditions existing in the tissues, and that Raper's reaction, which involves less specific oxidases, does not occur to an appreciable extent under conditions obtaining in the epidermis.

The action spectrum for the melanization of the epidermis is the same or very similar to the erythema spectrum (131, 177). Such an action spectrum could agree with either the absorption spectrum of protein, nucleic acid or tyrosine, and hence is compatible with any of the hypotheses that have been outlined in the preceding paragraphs. There have been numerous statements, however, to the effect that the "tanning" spectrum has a considerably longer wavelength limit (177, 250), and it is indeed true that when applied in comparable erythema dose, sunlight or carbon arc radiation, both of which are rich in wavelengths longer than  $0.32\mu$ , produce a deeper tan than mercury arc radiation which is weak in such wavelengths.

An explanation for this apparent discrepancy was provided a few years ago in studies by Henschke and Schultze (131, 132), which seem to have attracted relatively little notice. They observed, as had I. Hausser a year earlier (123), that a dark brown coloration of the skin is brought about by wavelengths longer than those that produce erythema, and made a thorough study of the characteristics of this phenomenon (131, 132). This response, which undoubtedly represents the darkening of preformed melanin and which will be referred to herein as *pigment darkening*, differs in several respects from the primary melanization that follows sunburn. The action spectrum for pigment darkening extends from about  $0.30\mu$  to  $0.42\mu$ , with a broad maximum near  $0.34\mu$  (curve PD, fig. 3), thus, in contradistinction to erythema and pigment formation, it is readily brought about by sunlight passing through window glass, which removes wavelengths shorter than  $0.32\mu$ . Pigment darkening may appear within the first few minutes

of exposure to sunlight, and usually reaches its maximum within an hour, whereas melanization is not manifest until a few days later. Several-hundred fold greater dosage of radiant energy is required to bring about pigment darkening than is required to cause erythema and melanization. Pigment darkening is most pronounced in skin that has been previously sunburned and still retains traces of suntan, whereas melanization is most pronounced in skin not previously sunburned. Pigment darkening does not occur if  $O_2$  is removed by blanching the skin by pressure with a quartz plate, whereas erythema and melanization are not affected by this treatment. Histological examination shows that these longer wavelengths do not cause pigment migration, nor the formation of new melanin (115).

It is probable that the tanning spectrum described by Luckiesh and Taylor (177) represents a combination of the action spectrum of this pigment darkening process with that of melanization, since those investigators did not differentiate the two processes.

Miescher and Minder (200), who confirmed and extended the findings of Henschke and Schultze, point out that either ultraviolet radiation (171, 188) or heat (170, 171) brings about darkening of pigmented cadaver skin, and that this seems entirely comparable to the pigment darkening in the living tissue. Miescher and Minder, who showed that all three phenomena take place only in the presence of  $O_2$ , believe that a common mechanism is involved, thus they suggest is the oxidation of pigment already present in the skin in a reduced leuco-form. It is possible that the "melanoid" pigment described in human skin by Edwards and Duntley (84) as a result of studies of spectral reflection, is a leuco-form of melanin, or a mixture of the oxidized and reduced forms. Pigment darkening has been indicated in the scheme in figure 2, as a photochemical reaction directly involving the melanin in the epidermis, the leuco-form of melanin might well be the light absorber for this process.

Henschke and Schultze (132) showed that a relatively large proportion of the suntan produced by sunlight may be due to pigment darkening, because of the great proportion of wavelengths between  $0.30\mu$  and  $0.42\mu$  as compared to those shorter than  $0.32\mu$  (see figs 1 and 3). This should be particularly true when the sun is far from zenith as in late afternoon or early morning, and at noon in late fall or early spring in temperate latitudes. It is possible that the pigment darkening effect accounts for the fact that many individuals retain a relatively dark suntan throughout the winter months, and the absence of this process may be an important factor in the pallor that develops in those seldom exposed to sunlight, a condition too frequently taken to indicate anemia.

Darkening of previously sunburned areas of the skin can be brought about by the administration of sex hormones (86, 112, 113), or other agents (146). The mechanism underlying this change in color has not been elucidated, it may represent either melanization or pigment darkening. The phenomenon is of particular interest because it illustrates the complexity of suntan, and of the factors that determine skin color.

*Acquired immunity to sunburn.* It is commonly recognized that the skin

becomes more resistant to sunburn after an initial exposure to sunlight. Some time just prior to 1900 (101), Finsen performed an experiment to determine the cause of this acquired immunity, his interpretation of which was so seemingly conclusive that it was at once accepted. As a result, a false concept was engendered which still persists. Finsen covered a part of the arm with India ink, leaving the remainder uncovered, and exposed the whole arm to sunlight for 3 hours. The uncovered part was sunburned, but the covered part remained unchanged. After several days, when the sunburned area of the arm had become tanned, he again exposed the arm to sunlight without any covering. After this second exposure, that area developed sunburn which had been protected by the ink during the first exposure, whereas the previously sunburned areas were scarcely affected. Finsen attributed the acquired immunity to screening by pigment developed as a result of the first exposure, which he thought mitigated the effects of the sun's rays by absorbing them. The idea that suntan directly protects against sunburn was generally accepted, and is widely held today.

About 1920, however, investigators began to point out that immunity to sunburn does not closely parallel the amount of visible suntan (152, 195, 217, 235, 236, 258), and it is now clear that melanin can play only a secondary rôle in acquired immunity to sunburn. Particularly convincing proof that such immunity may be acquired without any pigmentation whatsoever comes from two sources. The non-pigmented areas of the skin of vitiligo patients become more resistant to ultraviolet radiation as a result of exposure to that agent (195, 258), without any melanization occurring in these areas, and albinos, who do not form melanin, also become less sensitive after exposure (174, 236).

Observations by Guillaume in 1926 (111) indicated that relative immunity to sunburn is conferred by thickening of the horny layer of the epidermis. This finding was supported by Lovisati in 1929 (174), and in 1930 Miescher (196) carried out a thorough histological study, showing that marked thickening of the corneum occurs as a result of the epidermal hyperplasia brought about by the action of ultraviolet radiation. Measurements of the transmission of the ultraviolet radiation by the corneum, since obtained (see p. 487), show that this layer is very opaque to the wavelengths that cause sunburn, so that small increases in thickness should provide markedly increased protection for the living epidermal cells. The extreme resistance of the palms of the hands and the soles of the feet, which have little pigment, is no doubt due to the very thick layer of corneum they possess. Schall and Alius (235, 236) found that the maximum reduction of sensitivity of sunburned skin appears about one week after the exposure, and that normal sensitivity is regained in about 50 to 60 days. Hyperplastic thickening of the epidermis might reach its maximum in the former period, and normal thickness be regained by the end of the latter, suggesting that thickening of the corneum is the major factor in conferring immunity. Suntan, on the other hand, may persist for many months after the immunity is lost.

While all this evidence indicates that thickening of the corneum is the major factor determining the relative immunity to sunburn following exposure, the possibility that melanization and other factors play a rôle cannot be categorically

denied. The amount of radiation transmitted by the corneum is a function not only of the thickness, but of the attenuation coefficient which in turn is a function of both absorption and scattering (see p 487) Thus, the transmission of the corneum might be altered in three different ways (a) by changes in the absorbing components, e g , the accumulation of melanin, (b) by thickening, which results from epidermal hyperplasia, and (c) by alteration of the scattering characteristics. As pointed out above, the first of these factors is no doubt the most important, the relative importance of the second and third is more difficult to assess. Melanin is deposited largely in the basal-cells of the epidermis of the white races, although some of it migrates into the more superficial layers (see p 498). Melanin absorbs the sunburn radiation strongly (27), and should afford a certain degree of protection whether in the corneum or in the malpighian layer itself, where it may act as an "internal filter". In the skins of negroes, the pigment is much more uniformly distributed throughout the epidermis than in the skins of the white races, and this, no doubt, accounts in part for the relative immunity of the negro to sunburn (198), although it is also possible that the corneum is thicker in the negro race than in the white. Migration of melanin into the more superficial layers may be an important factor in conferring relative immunity to sunburn during the first few days following an exposure, before the corneum becomes appreciably thickened. Observations by Hausmann and Spiegel Adolph (127) suggest that alteration of the proteins of the corneum by ultra violet radiation may decrease the scattering, and hence the transmission by that layer. It is conceivable that the transmission of the corneum might be influenced by its moisture content, since the amount of water between the elements of which it is composed may affect the scattering function. Measurements by the writer have shown, however, no appreciable effect on the erythema threshold to result from the application of water to the skin's surface, nor from sweating (43). The latter finding is somewhat surprising, since components of both sweat and sebum absorb the sunburn producing radiation to some extent (68, 197).

Development of immunity to ultraviolet radiation by the epidermal cells themselves was first suggested by Perthes (217). Strong evidence to the contrary is to be found, however, in experiments by Miescher on the cornea of the rabbit eye. When exposed to ultraviolet radiation at regular intervals over a 28 week period, the sensitivity of the cornea to that agent did not change (197). The cornea does not possess a layer comparable to the corneum, to thicken and thus protect the cells of the corneal epithelium. Perthes (217) based his belief in cellular immunity on the apparent rapidity with which sensitivity to sunburn decreased after exposure. He found that a second dose of ultraviolet radiation produced less effect when it fell on a spot already exposed to that agent, even so short a time as one hour previously. Schall and Alius (235) showed, however, that incomplete summation of fractionated doses may lead to erroneous conclusions in such experiments, and may account for Perthes' finding and numerous other conflicting statements regarding immunity to sunburn that are to be found in the literature. Schall and Alius believe, however, that decreased sensitivity

becomes more resistant to sunburn after an initial exposure to sunlight. Some time just prior to 1900 (101), Finsen performed an experiment to determine the cause of this acquired immunity, his interpretation of which was so seemingly conclusive that it was at once accepted. As a result, a false concept was engendered which still persists. Finsen covered a part of the arm with India ink, leaving the remainder uncovered, and exposed the whole arm to sunlight for 3 hours. The uncovered part was sunburned, but the covered part remained unchanged. After several days, when the sunburned area of the arm had become tanned, he again exposed the arm to sunlight without any covering. After this second exposure, that area developed sunburn which had been protected by the ink during the first exposure, whereas the previously sunburned areas were scarcely affected. Finsen attributed the acquired immunity to screening by pigment developed as a result of the first exposure, which he thought mitigated the effects of the sun's rays by absorbing them. The idea that suntan directly protects against sunburn was generally accepted, and is widely held today.

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While all this evidence indicates that thickening of the corneum is the major factor determining the relative immunity to sunburn following exposure, the possibility that melanization and other factors play a rôle cannot be categorically

denied. The amount of radiation transmitted by the corneum is a function not only of the thickness, but of the attenuation coefficient, which in turn is a function of both absorption and scattering (see p 487). Thus, the transmission of the corneum might be altered in three different ways: (a) by changes in the absorbing components, e.g., the accumulation of melanin, (b) by thickening, which results from epidermal hyperplasia, and (c) by alteration of the scattering characteristics. As pointed out above, the first of these factors is no doubt the most important, the relative importance of the second and third is more difficult to assess. Melanin is deposited largely in the basal-cells of the epidermis of the white races, although some of it migrates into the more superficial layers (see p 498). Melanin absorbs the sunburn radiation strongly (27), and should afford a certain degree of protection whether in the corneum or in the malpighian layer itself, where it may act as an "internal filter". In the skins of negroes, the pigment is much more uniformly distributed throughout the epidermis than in the skins of the white races, and thus, no doubt, accounts in part for the relative immunity of the negro to sunburn (198), although it is also possible that the corneum is thicker in the negro race than in the white. Migration of melanin into the more superficial layers may be an important factor in conferring relative immunity to sunburn during the first few days following an exposure, before the corneum becomes appreciably thickened. Observations by Hausmann and Spiegel Adolph (127) suggest that alteration of the proteins of the corneum by ultra-violet radiation may decrease the scattering, and hence the transmission by that layer. It is conceivable that the transmission of the corneum might be influenced by its moisture content, since the amount of water between the elements of which it is composed may affect the scattering function. Measurements by the writer have shown, however, no appreciable effect on the erythema threshold to result from the application of water to the skin's surface, nor from sweating (43). The latter finding is somewhat surprising, since components of both sweat and sebum absorb the sunburn producing radiation to some extent (68, 197).

Development of immunity\* to ultraviolet radiation by the epidermal cells themselves was first suggested by Perthes (217). Strong evidence to the contrary is to be found, however, in experiments by Miescher on the cornea of the rabbit eye. When exposed to ultraviolet radiation at regular intervals over a 28 week period, the sensitivity of the cornea to that agent did not change (197). The cornea does not possess a layer comparable to the corneum, to thicken and thus protect the cells of the corneal epithelium. Perthes (217) based his belief in cellular immunity on the apparent rapidity with which sensitivity to sunburn decreased after exposure. He found that a second dose of ultraviolet radiation produced less effect when it fell on a spot already exposed to that agent, even so short a time as one hour previously. Schall and Alius (235) showed, however, that incomplete summation of fractionated doses may lead to erroneous conclusions in such experiments, and may account for Perthes' finding and numerous other conflicting statements regarding immunity to sunburn that are to be found in the literature. Schall and Alius believe, however, that decreased sensitivity



first appears as early as twelve hours after exposure—too early to be accounted for by either migration of melanin or hyperplasia. Development of immunity at such an early period after exposure is difficult to explain.

Many ointments and lotions have been devised to provide artificial protection against sunburn, but these vary widely in their effectiveness. Any material capable of absorbing radiation between  $0.29\mu$  and  $0.32\mu$  should afford such protection, but there are numerous other additional factors, e.g., the degree of scattering, which determine the effectiveness. There is variation in the protection afforded to different individuals by a given preparation, and this is related to the individual erythema threshold (43). Window glass and some plastics afford good protection against sunburn.

*Quantitative aspects.* The degree of erythema developed in response to a given dose of ultraviolet radiation is a function of a number of variables, many of which have been discussed in preceding pages. As a result, quantitative measurements and comparisons are subject to considerable uncertainty. The importance of the transmission of the corneum in determining the amount of radiation reaching the living cells has already been discussed at length, the sensitivity of the living epidermal cells themselves to ultraviolet radiation, and the sensitivity of the minute cutaneous vessels to the dilator substance, are factors that might be expected to vary as well (43). The course of development of the erythema varies from individual to individual (233), and this renders the erythema threshold—measured at a given interval after exposure, according to the usual procedure—an arbitrary index at best. When the complexity of sunburn is considered, it is not surprising that many conditions, external and internal, affect it. A number of these have been discussed in preceding pages, and a few more may be mentioned.

There have been numerous studies to determine the effect which heating the skin may have on sunburn, the results of which often appear contradictory. Investigations by Schall and Alus (234) and by Clark (57) indicate that during the exposure an increase in temperature has little effect on the erythema threshold, as might be expected for a photochemical reaction, but shortens the time to appearance of the erythema. Confusion in previous studies may have resulted from failure to separate these two aspects of the response.

Sulfanilamide, when injected into the skin in high concentrations, lowers the erythema threshold (30, 99) probably by altering the susceptibility of epidermal cells to the radiation. Morphine injected intradermally produces the opposite effect, but in this case by absorbing the sunburn producing radiation (227). No doubt other drugs affect the erythema threshold. Sensory (4, 226) or sympathetic (203) denervation alters the response to sunburn-producing radiation, probably due to changes in vascular response.

The erythema threshold varies considerably among individuals, in the same individual from time to time, and from one skin area to another, adding to the difficulty of comparative study. It is common belief that there is a close relationship between complexion and susceptibility to sunburn, red blonds being considered most susceptible, dark brunets least, but although there is certainly

a general relationship of this kind between complexion and the erythema threshold (97), anyone who makes measurements of a large number of individuals must be struck by the frequent exceptions to any rule. Certainly the thresholds of negroes of pure lineage are much higher than those of whites (124, 198).

Ellinger (97) finds characteristic seasonal changes, the threshold falling during the summer, he also attempts to correlate the erythema threshold with the physical state of the individual, particularly with the activity of the thyroid gland.

The erythema threshold is not necessarily an accurate measure of the amount of discomfort an individual may experience as the result of a given dose of sunburn producing radiation, since there may be marked qualitative differences in the response of individuals. Even among those who respond so abnormally to sunburn that they are diagnosed as photosensitive, the threshold is often within the normal range (29, 126).

Quantitative and qualitative differences in individual response account for only a part of the apparent vagaries of sunburn as they appear to the casual observer, who is often unaware of the great variations in intensity of the sunburn producing wavelengths of sunlight. Those wavelengths shorter than  $0.32\mu$  constitute less than 0.2 per cent of total sunlight (see fig. 1), and a most variable part of it. This component is subject to much greater change with the amount of atmosphere through which the sun's rays pass than is the visible component, because of greater attenuation by the atmosphere (p. 485), and hence the former varies much more with time of day, latitude, and season. It is only natural to evaluate the intensity of sunlight in terms of light perceived by the eye, but this organ does not record the intensity of the sunburn producing radiation—thus the latter may be overestimated or underestimated at one time as against another. Sky radiation, i.e., scattered solar radiation from the sky, is another confusing factor. At noon on a very clear day in temperate latitudes, the sky radiation constitutes only about 10 to 15 per cent of the visible component falling on a horizontal surface, whereas for wavelengths shorter than  $0.32\mu$ , the direct and sky radiation are about equal (178, 218), obviously one need not be directly exposed to the sun to receive a sunburn if he is sufficiently exposed to the sky. On a lightly overcast day, particularly in a fog, the scattered sunburn producing radiation may be many times the direct, and one may, to his surprise, be sunburned under such conditions. At high latitudes at midday, the sunburn producing component of the sky radiation is also much greater, relative to that of the radiation coming directly from the sun, than at low latitudes (60).

Another factor not generally recognized is that slight amounts of smoke or dust that are not particularly noticeable to the eye, may wipe out the wavelengths shorter than  $0.32\mu$ , and smoke polluted fog may provide very good protection against sunburn. Reflection from snow or ice may be an important factor, as is testified by the terms "snow burn" and "glacier burn", reflection from water seems to be less important (64). These variables probably account for many false impressions regarding sunburn.

**Cancer** The evidence that sunlight is a major factor in or even the pre-

dominant cause of human cutaneous cancer, has been considerably strengthened by the results of recent experimental and epidemiological studies. The concept itself is not new, having been introduced at about the turn of the century. Earlier studies consisted entirely of attempts to correlate clinical findings with extent of exposure and susceptibility to sunlight, but subsequent to 1928, when Findlay first showed that cutaneous tumors of mice and rats could be induced by exposure to mercury arc radiation, experiments have been numerous. At present, five major lines of evidence support the concept: (a) cancer of the skin occurs principally on the parts of the body most exposed to sunlight (about 95 per cent on the face and hands), (b) cancer of the skin is more common among outdoor than among indoor workers, (c) the incidence of cancer of the skin is greater in regions of the earth that receive high insolation, (d) cutaneous cancer is less prevalent in negroes than in the white races, presumably because the former are less susceptible to sunlight, and (e) cancer of the skin of laboratory animals (mice and rats) can be induced by exposure to ultraviolet radiation. The writer reviewed the then available evidence on these points in 1940 (28), and will confine the present discussion largely to more recent findings.

Close relationship between the sunburn and carcinogenic mechanisms is suggested by the fact that both have the same long wavelength limit at about  $0.32\mu$  (28, 104, 223, 232). Hence, it seems reasonable to relate the carcinogenic mechanisms to the same fundamental injurious action on cells that is associated with sunburn, and is characteristic of ultraviolet radiation of wavelengths shorter than this limit. The attractive alternative hypothesis has been offered, that the ultraviolet radiation brings about somatic mutations by direct action on the nucleic acid of the chromosomes (181). Supporting this hypothesis is the fact that ultraviolet radiation is very effective in inducing mutations in unicellular organisms, (140) and in sex cells of *Drosophila* (71, 181), the action spectra of the former indicating that nucleic acid is the light absorber. The difficulties encountered in determining the true action spectrum for tumor induction are, however, even greater in this case than in that of sunburn, and it is not likely that a choice can be made between the two hypotheses on the basis of such measurements (32). It has been suggested that ultraviolet radiation acts on the steroids of the skin to produce a chemical carcinogen, but recent evidence indicates that this is very unlikely (22, 38). The chronic irritation hypothesis of cancer induction—less popular today than in the past—receives some support from the chronic "precancerous" changes that are associated with human cutaneous cancer. However, although chronic changes also occur in mice that are exposed to ultraviolet radiation, these changes are not necessarily preliminary to tumor formation since there is evidence that tumor cells are actually present very early in the course of the exposures, possibly immediately after the first exposure (34, 37). Other chronic lesions of skin have also been attributed to continual exposure to sunlight (126).

In writing the above paragraph the findings on the mouse were applied to man without hesitation. It is implicit in this application that the long wavelength limit for carcinogenesis would be the same for man as that found ex-

perimentally for mice and rats, a likely relationship, since the same long wave length limit is characteristic of a large group of injurious biological effects (p 491). The validity of the assumption is a matter of some importance, particularly in considering the geographical distribution of human cutaneous cancer, and hence an apparent discrepancy and its cause need mention. Malignant tumors of human skin are virtually all epithelial, i.e., carcinomas, but in apparent contrast to this, sarcomas—non-epithelial tumors—predominate among the tumors induced in mice by ultraviolet radiation (107). This apparent lack of agreement between the case of man and that of the mouse is explainable in terms of differences in penetration of the ultraviolet radiation into the skin of the two species (155). Those wavelengths shorter than  $0.32\mu$  are virtually all absorbed in the epidermis of man, and it is here, where only epithelial cells are present, that human cutaneous cancer appears. In the mouse, on the other hand, this radiation penetrates much deeper, to reach connective and other non-epithelial tissues, accounting for the high proportion of sarcomas found among the tumors induced by ultraviolet radiation in these animals.

Until recently, data on incidence of human cutaneous cancer with respect to geographical latitude have not been too convincing, but recent studies by Dorn (77) indicate a clear north-south distribution. This investigator has made a careful analysis of the incidence of various types of cancer occurring in certain regions of the United States, which he groups as North, West, and South, but which actually form a series with respect to latitude, the weighted averages being  $41^\circ$ ,  $38^\circ$ , and  $32^\circ$  for north, west and south respectively. Dorn's findings are particularly striking in that cancer of the skin and cancer of the buccal cavity (largely cancer of the lip), show a clear-cut north and south distribution. For example, the incidence of cutaneous cancer in the white male populations was 23.1, 48.5, and 116.4 per 100,000 for north ( $41^\circ$ ), west ( $38^\circ$ ), and south ( $32^\circ$ ), respectively. Similar distributions occur for white females and for cancer of the buccal cavity for both sexes. Such a north-south distribution was not found for cancer occurring at other sites in the body, and hence it appears that some factor related to latitude is involved in the occurrence of tumors of the exposed parts, the skin and the lip.

On first consideration the wide variation of cutaneous tumor incidence with latitude seems difficult to account for, because one is apt to think in terms of visible sunlight, which does not vary so greatly with latitude (see p 485). Ultraviolet radiation shorter than  $0.32\mu$  varies much more widely with latitude, however, as reference to figure 1 and equation (1) will illustrate. The values of  $m_\lambda$  in equation (1) are much greater for the sunburn producing radiation than for that perceived by the eye, which means that the former will vary much more with the zenith angle of the sun, and hence with latitude and time of day, than visible radiation. The variation is not great enough, however, to account alone for the variation in incidence of cutaneous cancer with latitude. Experimental findings on mice help to explain this discrepancy, since (a) intensities of ultraviolet radiation below a certain critical value have relatively little effect (33), which would mean that the length of the day during which this agent can

be effectively carcinogenic is limited to the time during which the intensity is maintained above a critical limiting value, and (b) the time between exposures to ultraviolet radiation is a very important factor in tumor induction (35, 41), rest periods permitting partial or complete recovery (34, 37). With increasing latitude the number of days during the year on which the intensity of the radiation is above the critical value decreases, and thus there will be a longer rest period each year during the winter months which should lead to much slower development of cutaneous tumors. An exact quantitative analysis is not feasible, but all these factors would tend to increase the influence of latitude on cutaneous cancer incidence.

Dorn's data also indicate a lower incidence of skin cancer in negroes as compared to the white population (77).

None of the five arguments presented is completely convincing in itself, but as the evidence accumulates, it converges in a striking manner to support the conclusion that sunlight is the major cause of cancer of the skin. Lest this conclusion should appear too alarming to some reader, the writer hastens to point out that cancer of the skin affects only about two-tenths of one per cent of the population under the most severe condition represented in Dorn's data, and that as a whole, cutaneous cancers are among the least malignant of cancers, with the exception of certain highly malignant, but rare, types that are probably not caused by sunlight.

**Vitamin D.** The formation of vitamin D, and the resultant antirachitic action, is the one clear cut, beneficial effect known to be produced by sunlight impinging upon human skin. Its relative importance would, therefore, seem to merit a more prominent position and greater space in this review, but most aspects of the subject are so well known, and have been so adequately treated elsewhere that extensive discussion would be superfluous.

Vitamin D is formed by the action of ultraviolet radiation of wavelengths shorter than approximately  $0.32\mu$  upon 7-dehydrocholesterol or some very similar precursor steroid compound. The fact that this process has the same approximate long wave length limit as sunburn does not indicate any relationship between the two processes, and may be regarded, from a physiological point of view, as fortuitous. The action spectrum for antirachitic action has been determined for rats by Bunker and Harris (50) and by Knudson and Benford (156), and while the two sets of data differ to some extent, both indicate that the photochemical mechanism is definitely not the same as that of sunburn. Knudson and Benford's measurements of the action spectrum agree closely, and those of Bunker and Harris reasonably well, with the absorption spectrum of 7-dehydrocholesterol (36). This agreement suggests that the photochemical reaction takes place near the surface of the skin where there is little or no shielding by intervening layers as in the case of sunburn, and hence that the site of formation of vitamin D is probably in the corneum rather than in the living cells of the epidermis. That this is the site of formation is also indicated by the fact that a considerable amount of vitamin D is removed in bits of desquamating corneum which may be rubbed or washed off from irradiated skin (129).

Attempts to correlate the geographical incidence of rickets with the incidence of sunlight have not met with great success partly because adequate measurements of the antirachitic part of sunlight have not been available, but also because the picture is complicated by the dietary factor. In this respect it is of interest to note that in Greenland, rickets is unknown among the Esquimaux (23), who certainly receive little antirachitic radiation.

**Photosensitization.** Quantitative and qualitative variations in susceptibility to sunburn have already been mentioned. When these deviations from the norm are sufficiently great they may be considered as pathological conditions, and are properly designated as *polymorphic light eruption*, the term indicating the variability of the responses included in this category (29, 126). Other idiopathic types of sensitivity to sunlight occur in which the sunburn mechanism is not involved, but these are rare (29), and lie outside the scope of the present review. In addition there are forms of sensitivity to sunlight induced by photosensitizing materials, including certain drugs, the photosensitizer entering the body by contact in some cases, by ingestion or injection in others (29).

There is a particular type of photosensitization, given the name *photodynamic action* which is of interest, here, chiefly because it is sometimes invoked in explanation of experimental findings to which it does not apply. This type of photosensitization is produced by the introduction of a wide variety of dyes or natural pigments into living systems. Destructive processes are then brought about when the sensitized tissues are exposed to light, which may be manifold in their expression but which have a common mechanism under the action of light, oxidation of readily oxidizable substances in the photosensitized system takes place with the uptake of oxygen, the effects usually are injurious. The role of the dye in this reaction is to capture light quanta and transfer the energy, so gained, to the reaction, the dye itself remaining chemically unchanged at the end (29).

The porphyrin pigments are among the active photosensitizers of the photodynamic type, and it has been thought that they play an important rôle in the physiological responses of the human body to light. There is no evidence for this, however, beyond the fact that massive doses of hematoporphyrin will, like numerous other photodynamic dyes, cause severe photosensitization if introduced into the blood stream. It is even doubtful that such relatively high concentrations of porphyrin as appear in the body in certain pathological conditions cause photosensitization, general belief to the contrary notwithstanding (29). Conclusive evidence against the idea that porphyrins have any rôle in the production of sunburn in normal individuals lies in the fact that the wavelengths to which they sensitize do not agree with those that cause sunburn (29), and that photosensitization of the skin by porphyrin is inhibited by the absence of  $O_2$ , as is characteristic of photodynamic action but not of sunburn (44).

Porphyrins derived from chlorophyll and certain other pigments found in particular species of plants are the cause of photosensitization in domestic animals, a matter of great economic importance in some regions of the earth, but there is little or no evidence that comparable conditions exist in man.

Photosensitization of the photodynamic type does occur in man in some instances due to drugs or industrial products (see 29)

There is an erroneous belief that photobiological effects produced by ultraviolet radiation may be brought about by longer wavelengths when a photosensitizer is present. For example, there have been a number of unsuccessful attempts to accomplish the cure of rickets by using a photodynamic dye and visible light (see 29, pp 118-120)

**Temperature effects.** The stimulation of cutaneous sensations by radiant energy impinging on the skin has been the subject of recent extensive studies by Hardy and his co-workers. Three levels of sensation are elicited by successively greater intensities of radiant energy, warmth, heat and pain. Maximum sunlight is of sufficient intensity (slightly less than  $0.03$  gram calories  $\text{cm}^{-2} \text{sec}^{-1}$ ) to elicit the sensation of warmth or heat, depending upon the part and extent of the body it strikes (119, 133, 134, 135), but never reaches the threshold of pain sensation (120). The amount of radiant energy required to elicit the sensation of pain must be exceeded by about ten times (120) before actual damage to the skin occurs, and hence the persistent erythema and pigmentation reported to occur from intense exposure to infrared radiation from other sources is not to be expected from the most intense sunlight.

The actual stimulus for cutaneous sensation produced by radiant energy appears to be the alteration of the temperature gradient in the superficial layers of the skin (133, 210). Effectiveness in accomplishing such a change in gradient varies with the wavelength of the radiation, visible and near infrared radiation ( $0.36\mu$  to  $3.0\mu$ ) being partially reflected, and hence less effective in terms of incident energy than far infrared ( $>3.0\mu$ )<sup>\*</sup> which is entirely absorbed (209). Moreover, the far infrared, because it is more strongly absorbed, is more effective in altering the temperature gradient in the superficial layers, and hence is a better stimulus for cutaneous sensation.

A comparison between white and negro skin made by Hardy and Oppel (209) is particularly interesting in this respect. Both types of skin absorb the far infrared completely and very superficially, and to such radiation the skins of the negro subjects were somewhat less sensitive than the skins of the white subjects, apparently because of an intrinsically less sensitive cutaneous sensory mechanism on the part of the former. On the other hand, negro skin is a better absorber of sunlight than is white skin (see table 1), and the negro subjects were found to have a threshold to incident sunlight somewhat lower than that of the white subjects.

Sunburn causes a general hyperesthesia of the exposed area, the skin becomes more sensitive to touch and to temperature, and a mild stimulus such as contact with clothing often elicits the sensation of itching. Shumacher (240) found that the pain threshold was lowered 24 per cent, on the average, by a moderately severe sunburn.

Even far infrared radiation that virtually does not penetrate below the skin's

\* These are the approximate spectral ranges of the radiation used by Oppel and Hardy (209).

surface can alter the temperature gradient in the skin to a considerable depth (163). Under ordinary room conditions the temperature of the skin surface is a few degrees below the interior of the body, the extent of the difference depending upon the temperature of the room, but when radiant energy having the intensity of sunlight impinges upon the skin surface this situation may be reversed. The gradient is greatly affected by the circulation, which tends to distribute the heat throughout the body. The importance of this factor is shown by occluding the circulation while the skin is exposed to radiation, the temperature of the surface layers then rising. The importance of the circulation through the peripheral blood vessels in determining the skin temperature is shown in the case of the erythema of sunburn—temperatures of the surface layers being raised considerably above those for non-erythematous areas in the same temperature environment (163). It has often been suggested that melanin pigment in the skin has an important effect upon the distribution of heat. Both the measurements of Keller (153) and of Laurens and Foster (163) show that pigment has no such effect on long wavelength infrared radiation, as might be expected from the fact that this radiation virtually does not penetrate below the skin surface. The visible and short infrared, of which sunlight is largely composed, are absorbed somewhat more superficially in heavily pigmented skins than in those meagerly pigmented (153, 163), but the influence on the temperature gradient would not seem to be great, as compared to that of the circulation.

Dilatation of the superficial vessels and sweating both provide channels of heat dissipation in hot environments, the latter being the most important in extreme conditions. The relative importance of the cutaneous sensory receptors in bringing about adjustments of these two functions, as compared to the central regulatory mechanisms stimulated by heating of the blood, is not altogether clear (see 19, 52). It may be pointed out that so far as the cutaneous sensation of warmth is concerned, sunlight is a considerably less effective stimulus than the radiation from a hot stove (209), and this together with the variation in the threshold in different areas of the body, would seem to render this sensory mechanism a rather inaccurate index of temperature relationships of the body as a whole. The mechanism of vasodilatation in the local areas exposed to heat, not pronounced as a rule in exposure to sunlight, has yet to be completely elucidated (e.g., see 103). Local heating of the skin is not very effective in producing local sweating, the control of this process being mediated chiefly through central mechanisms (160).

**Other effects.** As our knowledge of the few clearly established cutaneous responses to sunlight increase, there seems correspondingly less reason to credit the numerous claims for additional effects. Most evidence supports the view that wavelengths longer than  $0.32\mu$  have no specific photochemical effect on normal skin, other than the pigment darkening effect which extends to about  $0.42\mu$ . There have been reports, however, that ultraviolet wavelengths longer than  $0.32\mu$  may bring about erythema (124, 199), and there is also some evidence that such wavelengths affect living systems in general. Lethal effects on



bacteria and other lower forms brought about by the longer wavelengths of the ultraviolet, have been the subject of recent studies by Hollaender and his co-workers (139, 148), whose findings indicate an essentially different character for these effects than for those produced by wavelengths shorter than  $0.32\mu$ . The writer (29, p. 117-118) has suggested that such processes may represent photodynamic action, for which point of view there is some evidence in earlier literature. The possibility that such effects are common to all living systems must be entertained, but it seems improbable that they will prove to constitute an important cutaneous response to sunlight.

It is possible that sunlight may destroy photolabile vitamins, e.g., vitamin A, and riboflavin, in the skin, but it seems improbable that this is important in the bodily economy, particularly in the clothed man.

Hill (136, 137) believed he had shown that long infrared radiation impinging on the skin caused swelling of the mucous membranes of the nose ("nose closing effect"), whereas red or short wavelength infrared caused shrinking of these membranes ("nose opening effect"), but the ample studies of Dufton and Bedford (80) and of Winslow, Greenburg and Herrington (257) have not confirmed these findings. Nose closing appears to result from heating of the skin by any means, including sunlight (80). Some individuals are apt to sneeze when they go from dark interiors to the sunlight, a reflex which may be associated with this phenomenon or possibly with visual stimuli, but which does not seem to have been adequately explained.

Walthard and Sporn (253) describe changes in the chronaxie of skin exposed to "red", "blue" and "ultraviolet" light. Their data suggest that the red and blue effects (no doubt considerable infrared was included in the latter) were probably associated with temperature changes in the skin, while the ultraviolet effects, which appear to be of a different character, were associated with sunburn.

Ehrenwald (87, 88, 89) found that exposure of the skin to radiation from incandescent lamps, either directly or through glass filters, brought about certain postural changes. This effect, if substantiated, may be of interest with regard to the participation of cutaneous sensory mechanisms in postural reflexes, but Ehrenwald's contention that it represents a specific cutaneous sensory mechanism dependent upon the wavelength of the light, is not justified. The spectral energies were not properly evaluated, and there seems no reason to assume that any sensory mechanism other than thermal was involved. As pointed out in the preceding section, the intensity of thermal stimulation is to a certain extent a function of wavelength because of differences in absorption.

**EFFECT ON THE EYES** To examine the process of vision would be beyond the scope of this review. A brief discussion of injurious effects of sunlight on the eye is in order, however. A number of these have been claimed, some without factual support, the inadequacy of the evidence having been pointed out by Verhoeff and Bell (251). The paper which these authors presented in 1916 must be regarded as a classical work on the subject, although, to judge from the relative infrequency with which it is quoted, it does not seem to have received as wide recognition as it merits. In it are described critical experiments on most as-

pects of injury to the eye by sunlight, and by radiation from artificial sources. Only in minor details is reinterpretation in terms of more modern concepts necessary, and the general conclusions appear as acceptable today as when they were formulated.

Ultraviolet radiation of wavelengths that produce sunburn causes injury to the cells of the cornea and conjunctiva, which results in the condition known as photophthalmia. This condition is characterized by pain, visual disturbances and photophobia, with excessive secretion, edema, and purulent discharge according to the severity of the injury. Although there has been no attempt to determine a complete action spectrum, the long wavelength limit has been set approximately by Verhoeff and Bell at about  $0.305\mu$ . By analogy with sunburn and other effects of ultraviolet radiation it may be judged that the primary action is on the protein, or possibly the nucleic acid of the cells. As in the case of sunburn of the skin there is a long latent period between the exposure to ultraviolet radiation and the first symptoms. From these and other resemblances, there seems every reason to regard photophthalmia as sunburn of the eye. Unlike sunburn of the skin, however, sunburn of the eye does not confer protection against subsequent exposure (197). This condition results frequently from exposure to artificial sources of radiation, particularly welding arcs, but may also occur as a result of prolonged exposure to intense sunlight. "Snowblindness" or "glacierblindness" is generally attributed to this cause (251), but it seems probable that subjective visual effects also play a rôle therein. It is not improbable that some of the severe visual symptoms described are associated with the photophobia which is a part of the symptom complex of photophthalmia.

Photophobia, i.e., pain accompanied by attempts to avoid light when there is a sudden increase of intensity, is an accompaniment of numerous pathological conditions of the eye. The retina is apparently the receptor of the stimulus, since photophobia does not occur in blind eyes, and since the stimulating wavelengths are those that affect the retina (244). Siegwart (244) found that blind eyes did not manifest photophobia, but in cases of unilateral blindness where the consensual iris reflex was preserved, illumination of the normal eye resulted in pain associated with the blind eye. Siegwart (244) and Lebensohn (165) present evidence that the pain is associated with reflex movement of the iris muscles, and reflex dilatation of the vessels of the conjunctiva. The reflex changes which lead to the pain involve the trigeminal nerve, and are abolished by removal of the Gasserian ganglion. It would seem, thus, that photophobia is a normal response to too intense light, which is aggravated by pathological conditions of the conjunctiva.

A fairly high incidence of tumors of the eye occurs in mice and rats exposed to ultraviolet radiation (172, 223). Reasoning from the parallel case of cancer of the skin, one may suspect that sunlight plays a rôle in the induction of tumors of the superficial structures of the human eye (13, 161).

If a man looks directly into the sun for too long a time he develops a scotoma which may remain permanently, this phenomenon is generally called "eclipse blindness". The damage is undoubtedly due to heating of the retina at the point

at which the sun's image is focused, to a temperature that causes local tissue damage. About half the energy of sunlight reaching the retina is perceived by the eye, and about half lies in the infrared, the ultraviolet portion being negligible (40, 83, 121, 224). Hence the infrared, although it has no specific effect, cannot be disregarded in designing glasses for looking into the sun. In fact, glasses which cut off most of the visible radiation without comparable reduction of the infrared, as, for example, most deep red glasses, may be dangerous because they lead to a false sense of security. With the visual mechanism protected by a reduction of the intensity of those wavelengths that specifically stimulate it, the individual may look into the sun without discomfort, yet receive enough infrared radiation to burn the retina (83).

It is sometimes assumed that eclipse blindness should have higher incidence in tropical regions (98), but there is little reason for this to occur. The intensity of sunlight reaching the retina is only about 20 per cent less with the sun at  $60^\circ$  from zenith, than with the sun at zenith (40). Thus, so long as the sun is reasonably high in the heavens it is dangerous to look directly into it, no matter what the latitude, and it would seem necessary to account in some other way for any differences in the incidence of eclipse blindness in different geographical regions.

It is highly improbable that ultraviolet shorter than  $0.32\mu$  reaches the retina in sufficient quantity to have deleterious effect, since it is absorbed so strongly by the proteins of the ocular media. Some changes in the retina have been reported after intensive exposure to mercury arc radiation (81), but such dosage would not be received in exposure to sunlight. There have been numerous claims that the longer wavelength ultraviolet has harmful effects, but this has not been substantiated (243).

The high incidence of cataract among glass blowers is usually thought to be caused by the intense radiation from the glass blower's furnace. The mechanism by which the cataract is produced has been debated, however, heat, and specific effects of both ultraviolet and infrared radiation having been invoked. The belief that ultraviolet radiation is the exciting agent has been held by various workers (e.g., 51, 56) since Schantz and Stockhausen (237) first claimed that the radiation from the glass blower's furnace includes such radiation. Quantitative measurements of the spectrum of such a source by Vogt (252), indicate that it contains little or no ultraviolet radiation as is to be expected from its relatively low temperature (251). Hence, so far as glass blower's cataract is concerned, it seems unprofitable to debate the manner in which ultraviolet radiation may affect the lens. As regards the action of infrared, in which this source is rich, it seems that any effect produced on the lens is probably due to heating of that organ, but that the iris, rather than the lens, is the principal absorber of the radiation that causes the heating (16, 17, 121).

It has been reasoned, in view of the accepted etiology of glass blower's cataract, that sunlight may be a causal factor in senile cataract. Actually the two cases are far from parallel because of the great differences between the spectral distribution of sunlight and that of the radiation from the glass blower's furnace and the fact that the lens and iris are subject principally to diffuse rather than direct sunlight.

In contrast to the radiation from the glass blower's furnace, sunlight contains ultraviolet radiation of wavelengths shorter than  $0.32\mu$ . Such radiation, if intense enough, can produce injury to the lens epithelium and thus might lead to cataract formation, and there is also the possibility that ultraviolet radiation may affect the protein of the lens directly as suggested by Clark (56). The intensity of radiation of these wavelengths in sunlight is not high, however, and the amount that penetrates through the cornea and aqueous humor to reach the lens must be quite small.

Differences in the primary site of development of senile cataract as compared to glass blower's cataract also suggest a different etiology (251). While it is conceivable that sunlight may play a rôle in the development of cataract in some cases, since to quote Bourne (46)—“no doubt there are as many ways of producing cataract as there are to injure the lens”—it would seem unlikely that it is an important factor in most. The high incidence of senile cataract said to occur in certain countries near the equator is probably to be explained in terms other than high insolation (98).

**SYSTEMIC EFFECTS** It is difficult to estimate just which of the many systemic changes reported to be brought about by sunlight are specifically caused by the radiation, are of major importance, or are spurious. This uncertainty is due in many instances to lack of information regarding the intensity, dosage and spectral distribution of the radiation employed, and even when these factors are reasonably well described it is often difficult to assign a specific cause to a given effect. Because the interpretation of many of the reported observations is uncertain an exhaustive review of the literature will not be attempted, but only those points will be treated which are satisfactorily explained, or which have aroused particular interest.

**The solar heat load** Of the total solar radiant energy striking the body a certain fraction is reflected by the skin or by the clothing, the remainder being absorbed. The absorbed portion, which is here called the *solar heat load*, is composed of direct radiation from the sun, and reflected radiation from the sky and from the terrain. The relative and absolute values of these three factors vary widely according to the position of the sun in the heavens and with the position of the man with respect to the sun, as well as with the reflecting properties of the skin, clothing and terrain. Table 2 gives some estimated values for the solar heat load and its component parts under a limited number of conditions. Since certain assumptions have had to be made regarding the geometry of the man, and the reflection from the terrain, skin and clothing, the estimates are only roughly approximate (39). Reflection from the body has been assumed to be 43 per cent, a value found by Martin both for very blond skin, and for khaki cloth (183).

The relative magnitude of the solar heat load is perhaps best understood by comparing it with the metabolic heat load. The average of the values for the total solar heat load estimated in table 2 is about 40 kilocalories per minute or 240 kilocalories per hour. Thus the maximum solar heat load may be two or three times the resting metabolic heat load (about 96 kilocalories per hour) or about equal to the metabolic heat load in ordinary walking (about 205 kilocalories per hour).

calories per hour) Obviously this factor cannot be disregarded in considering the total heat load

The physiological importance of the solar heat load cannot be separated from the total heat load, of which it is a variable part. The total heat load for a body out of doors is the algebraic sum of heat gains and losses through a number

TABLE 2  
*Estimated solar heat load under various conditions\**

POSITION OF MAN	ZENITH ANGLE	SOLAR HEAT LOAD† KILO CALORIES PER MIN			
		Direct	Sky	Terrain‡	Total
Erect	0°	0 90	1 13	1 84	3 87
	60°	2 67	0 45	0 74	3 86
Prone	0°	3 84	1 13	0 76	5 73
	60°	1 54	0 45	0 30	2 29

\* From Blum (39)

† Under the following atmospheric conditions, 20 mm H<sub>2</sub>O, 2.8 mm ozone, 300 dust particles per cm<sup>3</sup>, and the assumption that 43 per cent of the total solar radiation is reflected by the body and clothing

‡ Albedo of terrain assumed to be 0.25

TABLE 3  
*Attempted thermodynamic balance sheet for a man marching at 8 miles per hour, ambient air dry, with temperature about 37° C, terrain at 60° C, and body surface at 37° C*

	kilocalories per hour
Metabolism	+265
Total solar heat load	+234*
"Black body" radiation exchange with terrain	+128*
"Black body" radiation exchange with heavens	-128†
Evaporation	-506‡
Convection and conduction	± ?§
Total	-7 ± ?

\* From Blum (39)

† Estimated by Blum (39) from calculations by Simpson (245)

‡ Based on an average value of 882 grams of water loss per hour for men marching under hot desert conditions (5). This factor is the one that must vary to maintain a balance if such is achieved

§ These factors should be relatively small under such conditions

of channels which are indicated in table 3. The values assigned in this table are very rough estimates for a set of conditions which would impose a relatively high degree of heat stress. The derivation of these values cannot be discussed here, and the reader is warned that they are inexact and that the close balance indicated is entirely fortuitous. The table was formulated and is presented here only to show that a trustworthy estimate of the relative importance of the solar

heat load can be made only in terms of a number of other factors, e g, "black body" radiation to the heavens, which are themselves subject to variation (39)

The systemic changes imposed by the solar heat load as such, are the same as those imposed by heat load from any other source, they involve, primarily, a dilatation of the cutaneous vessels, and the stimulation of sweating. If the load is sufficiently great the effectiveness of these mechanisms in dissipating the heat load may be inadequate and the body temperature then rises. If water supply is limited, severe dehydration may occur due to sweating and this may lead to circulatory collapse, due chiefly to decreased blood volume (5, 6)

*Circulatory effects* As has just been said, circulatory readjustments may be brought about as the result of increased heat load. There is also evidence that systemic circulatory changes result from sunburn. Laurens and his co-workers have carefully examined this subject in a series of studies which were summarized, up to 1933, in a monograph by that investigator (162). In these studies, high dosages of carbon arc radiation have been employed as a rule. In the dog, lowering of systolic and diastolic blood pressure follows exposure to dosages sufficient to produce persistent erythema, cardiac output is also decreased. In normal man the lowering of blood pressure is less in extent, and if anything, there is a slight increase in cardiac output (147). The fall in blood pressure can not be explained by changes in blood volume, and according to Laurens' view, is the result of general peripheral arteriolar dilatation, due to the release of a dilator substance into the blood stream, the local release of dilator substance in sunburn has been discussed previously. The presence of such a histamine-like dilator substance in the blood of dogs after exposure to carbon arc radiation, has been correlated with the falling blood pressure (157, 164). Graham (108) has found that blood pressure lowering following carbon arc exposure is accentuated in adrenalectomized dogs, and that injection of desoxycorticosterone acetate or adrenal cortical extract abolishes the blood pressure lowering effect. Since the effect of histamine is enhanced by removal of the adrenal cortex and antagonized by adrenal cortical substances, Graham's findings may be interpreted to indicate that blood pressure lowering is due to the elaboration of histamine following carbon arc radiation. On the other hand, Graham also finds (109) that capillary permeability, as demonstrated by passage of dye from the blood into the tissues, is increased following exposure to carbon arc radiation, both locally and in the body generally, and that adrenal cortical substances inhibit this effect. Menkin (191) has shown, similarly, that injections of inflammatory exudates, or of leucotaxine, produce general capillary dilatation, and that this is likewise inhibited by injections of adrenal cortex. These later observations suggest that leucotaxine is released into the circulating blood from the sunburned skin (see p 495), and that a generalized lowering of capillary permeability results, which may play a part in the blood pressure lowering effect. Weiss, Robb and Ellis (254) found that histamine does not produce in man the effect on blood pressure that characterizes its action in numerous other species, and that it is destroyed rapidly so that its concentration in the blood does not

rise to high levels (see also 225) Again we are faced with difficulty in attempting to identify the dilator substance associated with sunburn

It is possible that other systemic effects result from the release of other inflammation-substances, and it seems unlikely that the total picture is greatly different from that produced by very superficial burns resulting from other causes

Carbon arc radiation, which has been employed in most of these studies, contains wavelengths other than those that cause sunburn, being particularly rich in longer ultraviolet wavelengths in the region of  $0.39\mu$  where the characteristic cyanogen band emission occurs. The possibility of some specific effect of these wavelengths, which are also present in sunlight, must be entertained, but for the present there is no direct evidence that they play a part in the circulatory changes. The total intensity of the radiation used in some of these studies has been approximately equal to that of maximum sunlight, and hence the effects of increased heat load are not entirely excluded. These points need mention because some experiments have indicated that mercury arc radiation—which is poor in the long wavelength ultraviolet radiation and in total energy relative to sunburn producing wavelengths—is not as effective in eliciting such changes as is carbon arc radiation, which more nearly resembles sunlight (see 162)

As regards the formed elements of the blood, exposure to ultraviolet radiation seems to produce a slight increase in red cell, white cell and platelet counts, but there is disagreement among various reports, due probably to the lack of uniformity in the dosages and procedures employed (158, 162, 166). Release of Menkens' leucocytosis factor (190) from the inflamed skin might bring about an increase in white cells, and other changes in the formed elements may result indirectly from the inflammatory process.

*Metabolism and growth* The measurement of metabolism after sunburn, when the cutaneous vessels of a considerable surface area are dilated, may be subject to error and misinterpretation (79), and there have been conflicting reports in this regard (162). Recently, Lippman (173) has reported a slight increase in basal metabolism, as measured by indirect calorimetry as had Lehmann (166) somewhat earlier. Lehmann (166) also described an increase in metabolism and in capacity for muscular exertion to accompany repeated exposures, but this seems to require confirmation. Release of fever producing substances from the site of inflammation in the skin may occur (193, 194). The production of vitamin D in the skin by ultraviolet radiation affects calcium and phosphorous metabolism, but beyond this there seems to be no clearly demonstrated specific influence of sunlight on metabolism (162).

No specific effect of sunlight on growth of human beings is known beyond the influence that vitamin D may have. Many experiments purporting to demonstrate effects of light on growth of laboratory animals have been described, but it is probable that many of these represent changes caused by abnormal conditions of temperature rather than specific effects of light. When small animals are placed in closed cages covered with glass and exposed to sunlight,

as has been done in many experiments of this kind, the temperature of the interior rises, due to what is commonly called the "greenhouse" effect. The greater part of sunlight is transmitted by the glass, and thus being absorbed by the walls of the cage is transformed into long wavelength "black body" radiation which cannot pass out through the glass. The result is that the temperature rises in the cage, and the animal is subjected to a large heat load by radiation from the walls of the cage to which must be added the heat load from the ambient air and from the direct rays of the sun. Forced ventilation of the cages does not ensure uniform temperature under such conditions, and may only add to the complexity of the situation. Rodents, the animals commonly used for such experiments, have poor ability to cool themselves by evaporation of water and also have low total heat capacity because of their small size. Thus they are poorly equipped to combat high temperatures, which may, therefore, affect their metabolism and growth. If different colored glasses are placed over the cages, as has been the practice in experiments of this kind, different temperature conditions must obtain inside the cages because of the different amounts of the sun's radiation absorbed by the glass. The conclusion that under such conditions one has demonstrated a specific effect of, say, red light, because animals have grown at a different rate or behaved differently in other respects under a piece of red glass than under glass of some other color, is not warranted. Effects on plants under similar conditions have obviously no relationship to those on animals, because light plays an entirely different rôle in the economy of the two kinds of organisms, yet there has been an unfortunate tendency to make this inference.

Recent experiments have shown that the suppression of growth of mice by repeated heavy doses of ultraviolet radiation is due to decrease of food intake (42), and it seems probable that this results indirectly from irritation of the animal's eyes (167). There seems to be no real evidence that sunlight has any direct effect on the growth of human beings enjoying adequate diets.

*Effects on endocrine systems* The sexual cycle of many kinds of birds and of certain mammals is influenced by light. It seems most probable that the eye is the receptor for the stimulus that brings about these changes, and that the pituitary is involved in mediating the effect (24, 59, 182). The principal effect of the illumination may be to prevent sleep, and to increase the activity of the animals (231). Certain experiments which appear to indicate that stimulating mechanisms other than the eye are involved (21) are open to objection as regards the methods used to measure and control the radiation. Recently, a hormonal mechanism has been proposed (47). It is occasionally suggested that a similar effect may occur in man (e.g., 168), and in support it is sometimes stated that the menses are suppressed in Esquimaux women during the months of the complete arctic night. This statement seems to come from observations by Cook (66), made during the first Peary North Greenland Expedition, but I have found no mention of such an effect by others who have observed the Esquimaux (e.g., 23, 221).

Ellinger interpreted certain of his experiments (95, 96) on mice to indicate



that excessive exposure to ultraviolet radiation brings about alteration of thyroid gland function, but no histological evidence of this has been found (42, 185)

Myerson and Neustadt (204) have reported that mercury arc radiation applied to the skin causes increased androgen excretion

*Therapeutic action* Curative effects of sunlight have been repeatedly described. These are usually attributed to the ultraviolet component, presumably those wavelengths shorter than  $0.32\mu$ , and it is generally assumed that the same effects are brought about by radiation from other sources rich in these wavelengths. Avitaminosis D can be cured by summer sunlight, or by radiation from other sources rich in wavelengths shorter than  $0.32\mu$ , but beyond its effect on this particular avitaminosis, clear cut examples of the therapeutic value of sunlight per se are conspicuously lacking. This statement should not be construed as a denial of the cures observed when sunbathing is included as a part of a regime used in the treatment of certain conditions, but only that the actual rôle of sunlight therein is not established beyond question. Moreover, one cannot completely disregard the numerous other claims for the therapeutic value of sunlight or ultraviolet radiation from other sources, particularly when one considers the success of the Finsen treatment of *Lupus vulgaris*<sup>6</sup>

In evaluating claims for curative effects of ultraviolet radiation, some papers dealing with prevention of the common cold merit discussion. Maughan and Smiley (184) reported 40 per cent reduction in the incidence of colds among subjects who received exposures to radiation from a mercury arc, as compared to control subjects who received no treatment. On the other hand Colebrook (65) and Doull, Hardy, Clark, and Herman (78) found very slight differences between exposed and control groups, the differences are probably not significant but both show fewer colds in the control than in the treated groups. Here are three studies, one of which indicates a significantly beneficial effect of ultraviolet radiation on colds, and two which show a slight detrimental, or probably no effect. The reason for the discrepancy may be sought in the mode of treatment, or in the criteria used for determining the incidence of colds. While the treatment was not identical in the three cases, one finds nothing here to account for the radical difference in the results. The criteria for determining incidence of colds are radically different, however, Maughan and Smiley having accepted the reports of the subjects themselves, whereas Colebrook, and Doull, et al depended upon objective examination. Diehl, et al (73, 74) made extensive studies of the effect of various drugs and of a placebo (inactive medicament) on the incidence of colds, using the subject's report as a criterion, and found that the placebo gave approximately the same decrease in incidence that was observed by Maughan and Smiley after treatment with mercury arc radiation. Thus there is reason to believe that the decrease in the incidence of colds which Maughan and Smiley reported was a "placebo effect", and that had a placebo

<sup>6</sup> The council for Physical Therapy of the American Medical Association (67) recognizes ultraviolet radiation as of possible value in the treatment of only a very limited number of conditions

<sup>7</sup> i.e., subjective improvement following the administration of an inactive medicament or treatment

been given to the control group, no difference in the incidence would have been observed. It may be mentioned that Colebrook used a very appropriate placebo in her studies, i.e., the same radiation filtered through glass to remove the wavelengths shorter than  $0.32\mu$ , and that she found no important difference between control, placebo, and treated groups under the conditions of her experiment. After considering this example, one cannot help wondering whether many of the other observed cures attributed to ultraviolet radiation are not of the same nature, for the widespread belief in its curative value renders it capable of exerting a strong "placebo" effect.

Bathing in the sunlight gives a pleasurable sensation of warmth when the ambient temperature is not too high or too low, and might be of value in convalescence, even though there is no specific effect of the sunlight. The pleasurable sensation no doubt comes from warming of the skin, and should not be greatly reduced by interposition of window glass, which at the same time would preclude the danger of severe sunburn.

*Heat stroke* An extended discussion of heat stroke would not be possible in this review, but cannot escape mention since the solar heat load may be an important part of the total heat load, and hence may play a part in this syndrome, at times. Presumably hyperthermia is the direct cause in many instances (75, 261). Recent studies by Adolph and his collaborators (5, 6) indicate that under conditions of excessive dehydration, circulatory collapse finally results from reduction of the volume of the circulating blood to a dangerous level. In hot environments dehydration occurs from sweating if enough water is not imbibed to prevent the blood volume reaching a dangerously low level.

The vasodilatation that occurs in the skin as a result of sunburn could be an exacerbating factor in cases of heat stroke involving exposure to the sun, since it should act in the same way as reduction of blood volume, and hence contribute to circulatory collapse. On the other hand, the superficial vasodilatation might result in greater heat loss by radiation. The importance of these factors is difficult to assess.

It is possible that there are some direct specific effects of sunlight in heat stroke, but such have not been demonstrated. It has been thought that sunlight impinging directly upon the head may raise the temperature of the meninges and brain, because hemorrhages have been found in these structures after death associated with excessive exposure to sunlight (82, 241, 247). Experiments carried out by Aron (12) in the Philippine Islands do not support this belief, however. This investigator found that monkeys fully exposed to bright sunlight succumbed in one to two hours, hemorrhages of the brain being found at autopsy, but that if only the head was exposed the animals survived long periods of insolation. Apparently the total heat load was the lethal factor rather than heating of the cranium. Failure of the mechanism of heat control, e.g., due to a neurological lesion, might predispose to heat stroke, and this would be most likely to occur when the subject was exposed to an unusual heat load, to which sunlight might be a contributing factor. In such a case, sunlight could easily be credited with a specific rôle it does not play. Until further evid-----

effects is forthcoming, it seems necessary to conclude that "sunstroke" is not essentially different from heat stroke

### SUNLIGHT AS A LIMITING ENVIRONMENTAL FACTOR

Sunlight is a major factor in man's environment. It not only influences the ambient temperature, but is directly responsible for the winds, the major ocean currents and rainfall, besides being the source of energy for all life processes. Thus, sunlight largely determines the areas of the earth that are habitable, but for the most part indirectly through the above functions—not by direct physiological action. Nevertheless, a great deal has been written about the deleterious effects of excessive sunlight in the tropics, and of the lack of sunlight in high latitudes, and it is in order, here, to consider how the direct physiological effects of sunlight might favor or hinder the inhabitation of various parts of the earth.

Although the solar heat load may be an important part of the total heat load, it need not affect life in tropical countries if habits are properly adjusted. The wearing of white clothing can considerably reduce the solar heat load (39)<sup>8</sup>, and outdoor exposure at midday can be avoided under ordinary conditions. Escape from the solar heat load should be an easier matter in the tropics than escape from the excessive heat load in some industrial plants, e.g., steel mills, in temperate climates (75, 128). Actually the intensity of the total radiant energy of sunlight, and hence the solar heat load, does not vary nearly as much with latitude as is commonly supposed. For example, reference to figure 1 will show that the difference in intensity with the sun at zenith (air mass 1), and with the sun at 60° from zenith (air mass 2) is not very great, amounting to about 20 per cent. With the sun at the summer solstice for the northern hemisphere, the intensity of the direct noon sunlight at the arctic circle is only a few per cent less than at the equator. At the north pole the total amount of sunlight received during 24 hours is greater at this time than at the equator because of the long summer day. Very hot days may occur in the arctic (e.g., 246), but the continuous recurrence of hot days and nights in the tropics no doubt constitutes a physiological and psychological stress which relatively short hot periods in the arctic do not achieve.

In contrast to the total intensity of sunlight, the intensity of the sunburn-producing component does vary greatly with latitude, due to the fact that the atmosphere absorbs strongly those wavelengths shorter than  $0.32\mu$  and hence the amount transmitted by the atmosphere varies greatly with the thickness of the atmospheric layer that must be traversed (see p. 505). The principal absorbing component of the atmosphere is ozone which varies in its distribution with respect to latitude and season, being present in least amount over the tropics where it remains approximately constant throughout the year, whereas in temperate latitudes it is present in greatest quantity during the winter months. This distribution of ozone tends to increase latitude and seasonal differences in

<sup>8</sup> The problem of proper clothing for hot environments is a complex one, involving numerous factors other than the reflection of solar radiation, e.g., see (261)

the sunburn producing component of sunlight. The marked difference in this component of sunlight could conceivably be a factor in determining the inhabitability of the tropics as compared to temperate or arctic regions, but that it is an important one seems improbable. The discomforts and dangers of sunburn, while not a factor to be disregarded, can be avoided by simple precautions under ordinary conditions, although under military conditions this may be more difficult at times. It seems probable that more persons are severely sunburned in regions outside the tropics than in them, for inadvertent overexposure seems more likely to occur when the temperature is low, because of failure to realize the extent of the exposure. The potential danger of developing cancer of the skin may be greatest in tropical regions for reasons previously discussed, and this has been represented as an argument against inhabitation of the tropics by fair skinned races, but while it seems a hazard that should be guarded against, it also seems unlikely that the increase in incidence of skin cancer in the tropics over that in temperate latitudes would affect enough of the population to serve as a real deterrent to colonization (see p 508). The danger could be largely avoided, of course, if it were recognized.

The antirachitic power of sunlight, since it results only from wave-lengths shorter than  $0.32\mu$ , must vary greatly with latitude, but deficiency in vitamin D can be so easily supplied in the diet or by direct administration of the vitamin, that it can hardly constitute a factor limiting modern colonization in any climate. There seems to be no good evidence that life in complete darkness affects the physiology of man to any great extent (162), provided his diet is adequate, and there seems likewise to be little evidence that the arctic period of total darkness imposes any important physiological effects due to the lack of sunlight *per se*.

It has been frequently alleged that the negro is better adapted to life in the tropics because his pigment protects him against sunlight. This is an old belief (e.g., 70, 142) which seems to stem from a misinterpretation of various observations, and to the teleological point of view with which the matter has commonly been approached. The following quotation from Perthes who more recently championed this idea (217) typifies the teleological attitude, "Wenn es nicht nützlich wäre, so wäre es nicht, phlegte von vielen Jahren der Physiologe Pflüger seinen Schülern bei der Erörterung dunkler Vorgänge im menschlichen Körper zu sagen".

One function that has been attributed to the negro's pigment is protection against the ingress of heat, but a glance at table 1 will show that a naked negro is actually at a disadvantage as regards the solar heat load because his skin reflects less of the incident solar radiation than does that of a naked white skinned individual. This additional heat the negro must get rid of by evaporation, which means that he must drink more water and produce more sweat under like conditions of exposure than must the white man. It has been suggested that the pigment of the skin serves to raise the surface temperature when exposed to sunlight, and thus to increase sweating which in turn helps to dissipate the heat load, but measurements of skin temperatures (153, 163) do not indicate

that pigment has any great influence on the superficial temperature gradient, and, moreover, sweating depends largely on internal rather than superficial temperature (160). Whatever the effect of pigment on superficial temperature the naked negro must be at a thermodynamic disadvantage as compared to the white man, since he absorbs more radiant energy, but any advantage or disadvantage in this respect would be virtually wiped out if the white man and the negro were both covered with the same kind of clothing.

The negro is less sensitive to sunburn and to cancer of the skin than is the white, and it is probable that the presence of considerable melanin pigment distributed throughout his epidermis is an important factor in determining both, but the advantage of this in competitive life in the tropics would appear to be relatively slight. As has been pointed out, sunburn can easily be avoided, and the proportion of cutaneous cancer among the population as a whole is small in any case.

Whatever the factors that account for the frequent success of the negro in supplanting the white man in the tropics, so carefully considered by Price (220), it seems most probable that the direct effects of sunlight have no great influence. On the other hand, it appears likely that the prolonged effects of constant hot environment, about the physiology of which we are not well informed, is an important factor in the "degeneration" of the white man in the tropics, but this is not a direct effect of sunlight.

Man now having added a third dimension to the "habitable" earth, one must consider the physiological effects of sunlight at altitudes as high as airplanes can operate. The increase in total solar radiation in going from the earth's surface to the outside of the atmosphere is not a very important factor, amounting to only about 35 per cent, as may be seen by comparing the curves for air mass 0 and air mass 1 in figure 1. The effect of the cold surrounding air and greater radiation to the heavens, makes cold rather than heat the important problem in high altitude flying. On the other hand the sunburn-producing ultraviolet radiation varies with altitude in a quite different manner and to a different extent, the ozone of the atmosphere, which is the component most important in cutting out this radiation, is stratified in its distribution, occurring in greatest concentration in the stratosphere. A number of measurements of the intensity of ultraviolet radiation at various altitudes indicate a considerable increase in the sunburn producing portion, beginning rather close to the earth (62, 76, 222), but the most complete measurements yet obtained, taken by O'Brien and his collaborators (206, 208) in connection with the stratosphere flight of the "Explorer II", indicates no appreciable ozone below about 15 kilometers, a height above the present limit of airplane operation. Whether or not there is an important increase in the incidence of sunburn-producing-radiation at present flying altitudes, and whatever the possibilities that the ceiling may be raised in the future, adequate protection should be readily obtainable by the use of plastics which do not transmit these wavelengths, or of window glass, in transparent enclosure surfaces.

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# THE PRESENT STATUS OF THE PROBLEM OF THERMAL BURNS

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The problem of thermal burns in its various aspects physiologic and therapeutic, was reviewed in a monograph by the author in 1942 which included the bulk of the pertinent literature up to December 1941. The present summary will cover the period 1942-1944 inclusive, except where previous writings have attained an increased importance or are subject to an altered interpretation in the light of recent developments.

Because some of the readers of this article may never have actually treated burns, the following composite case history can serve as a practical introduction to the review. In this case history certain of the physiologic problems, difficult or even completely unsolved, will be cited. The treatment used is generally adopted at the present time (January, 1945) and is an example of the present status of the treatment of burns.

*Composite case history* A man aged 30 years was admitted with flame burns of the arms, chest, upper abdomen and face. The accident occurred one hour before admission from a gasoline explosion. At the site of the accident the patient was wrapped in clean towels and brought to the hospital. On admission he moaned but when questioned stated that he suffered little pain. One-quarter grain of morphine was given intravenously in the fluid which was started in a foot vein. Before starting this fluid a specimen of blood taken from the same vein showed a hematocrit of 50. The fluid given was the so-called physiologic electrolyte solution (a mixture of two parts of normal isotonic—1/6th molar 0.9 per cent—saline solution with one part of normal isotonic—1/6th molar 1.75 per cent—sodium lactate solution. The final mixture was of the following concentration: sodium chloride—1/9th molar 0.6 per cent, 100 mM/L—and sodium lactate—1/18th molar, 0.6 per cent, 50 mM/L). While the physiologic electrolyte solution was running in 500 cc plasma was obtained and added to the infusion fluid. No shock was present.

At the same time as the above listed general treatment was begun definite local treatment was instituted. All those in the room with the patient wore masks and caps and those directly in attendance wore in addition sterile gowns and gloves. The burned clothes were cut away. The burn estimated by Berkow's formula to be 32 per cent of the body surface and a very minimal debridement was done with no additional anesthesia. Dirt was wiped away with soft cotton sponges using saline. No blisters were punctured and the entire burned area was covered with a four layer pressure dressing (1st layer vaseline gauze, 2nd layer sterile surgical squares, 3rd layer large amounts of mechanic's waste, and 4th layer elastic bandage or adhesive tape). This dressing was then left in place with no change until the fourteenth day.

The patient was sent to the ward and started on a routine of 200 cc physiologic electrolyte solution by mouth every hour on the hour. The hematocrit was redetermined four hours after the accident (3 hrs after admission) and found to be 57 despite the plasma and electrolyte previously given so 1000 cc. additional plasma was ordered. (Such a continued rise in the hematocrit despite minimal parenteral therapy is common and indicates the need for continued blood concentration determinations.) Sulfadiazine was given by mouth. During the first 24 hours the patient received 2000 cc plasma, 1000 cc whole blood, and 1500 cc of physiologic electrolyte solution intravenously and 4200 cc physiologic electrolyte solution by mouth. During the second 24 hour period no plasma was given but 500 cc

whole blood, 1000 cc physiologic electrolyte solution and 500 cc 10 per cent glucose were given intravenously while oral intake included 3400 cc physiologic electrolyte solution, fruit juices and water ad libitum, and the first attempts at a high caloric, high protein diet. The shock phase (1st 48 hrs) was thus passed without incident and the hematocrit did not arise above the reading of 59 obtained at the fourth hour.

The patient now entered the so-called toxic phase (48-120 hrs, occasionally as late as the third week). Physiologic electrolyte solution, 2400 cc per day, was kept up until on the fourth day a mild alkalosis ( $\text{CO}_2$  combining power of 70) caused a shift to be made to normal saline solution. (The eventuality of acidosis could be met by a shift to isotonic one-sixth molar sodium lactate solution by mouth.) Careful attention was paid to the urinary output and none of the other signs of burn toxemia (jaundice, stupor, delirium) developed. On the sixth day the hematocrit had decreased to 39 so more attention was paid to the high protein diet and several whole blood transfusions were given.

After the shock phase was over and coincident with the toxic phase, the patient entered the so-called septic phase. Sterile early definitive treatment plus oral sulfonamides, penicillin, maintenance of nutrition, and infrequent dressings, using mask technic, are the chief means of combatting such a condition.

The need for control of the phase of burn anemia and hypoproteinemia was especially accentuated when the first dressing of the burn was done on the fourteenth day. It was found that much (18 per cent of the body surface) of the burn was third degree and in the preparation for the inevitable and all-important skin grafting additional protein and hemoglobin were continuously lost from the exposed oozing surface. In places, granulations existed and in others sloughs which had to separate before the granulations would be ready for grafting. Infection, particularly with streptococci and pyocyanous organisms, was present, especially where the sloughs existed.

Grafting was begun on the 32nd day and completed by the 56th. During this time strict attention had to be paid to a high protein, high vitamin diet (as much as 300 grams protein per day), and to occasional whole blood transfusions to keep the plasma proteins above 6.0 grams/100 cc and the hemoglobin above 80 per cent. Once the grafted areas were entirely healed, fever, anemia, and hypoproteinemia disappeared promptly despite relaxation of control measures.

Several months later, when the patient was in good general shape, he returned for plastic improvement of one badly scarred healed area. This was done by substituting a thick graft for the thin scarred one already present.

In the remainder of this paper, various questions raised by the composite case report are presented under 23 headings as follows:

### *I The Local Burn Lesion*

#### 1 Immediate trauma (1)

##### a Necrosis (2)

##### b Edema (3)

##### Compression treatment (4)

##### c Increased lymphatic flow (5)

##### d Infection (6)

##### Rationale of early cleansing (7)

#### 2 Determination of the area and depth of the burn (8)

#### 3 Separation of burn sloughs (9)

#### 4 Healing and regeneration (10)

### *II The General Disturbances of the Burned Patient*

#### 1 Morphine and anesthesia (11)

- 2 Burn shock (12)
  - Plasma loss and plasma therapy (13)
  - Burn shock complicated by pulmonary damage (14)
- 3 Importance of environmental temperature in influencing the mortality following severe burns (15)
- 4 Burn toxemia (16)
  - The liver lesion and tannic acid (17)
- 5 Sodium therapy (18)
- 6 Burn anemia (19)
- 7 Burn hypoproteinemia (20)
  - Nutritional factors (21)
- 8 Metabolic factors (22)
- 9 Endocrine relationships (23)

Several of these items were more or less adequately covered by the previous review (1942). Others (especially nos. 4, 5, 7, 14, 18, 20, 21, 22, and 23) were not so satisfactorily presented in the 1942 book because of new developments since that time.

I *The Local Burn Lesion* 1 *Immediate trauma* Immediate trauma is important in burns. Locally it produces necrotic and inflammatory changes and may injure certain constituents of the blood flowing through and beneath the burned area. In some instances the blood may be hemolyzed and in others it may be heated to such a degree that generalized hyperthermia results. In still other cases the respiratory tract may be directly injured by heat or fumes. When severe enough the immediate trauma causes death. Most such cases never reach a hospital and in the mortality statistics they are listed as victims of conflagration. Other patients, more fortunate in that their immediate trauma is less severe and their resultant burns less extensive, live to go through the various phases discussed below. In such instances certain of the local effects of the burn are as follows:

2 *Necrosis* All deep burns are accompanied by actual destruction of tissue and it is this very fact which makes all forms of treatment imperfect. No method of therapy can restore the damaged cells to normal. The importance of the depth of the burn is recognized by the various classifications of severity of burns according to the penetration of the injury (Dupuytren, Boyer, Morton, etc., reviewed by the author, 1942). The reports of Elman and Lischer (1944), Leach, Peters, and Rossiter (1943), and of Prinzmetal, Bergman, and Hechter (1944) are of especial interest in this regard and consider the subject from a new standpoint. The St. Louis workers found three grades of damage to the skin: edema (produced by low temperatures or short exposure), wet necrosis (produced by medium temperatures and more exposure), and dry necrosis (produced by higher temperatures and more exposure). These authors pointed out that the type of necrosis may be more important than its depth. Their observations were made on anesthetized animals but were correlated with clinical observations. They finally presented evidence that wet necrosis, even though a less severe

injury than dry necrosis, may be more apt to lead to toxic and septic changes than the latter. Cellular infiltration, edema, and other signs of inflammation were noted in the wet necrosis type of lesion and not in the dry necrosis.

The Oxford group of Leach, Peters, and Rossiter (1943) in an excellent paper also considered the effect of the depth of the burn on general bodily changes. With a special water heated burning iron, these authors applied graded temperatures of 45 to 80° C to shaved anesthetized guinea pigs, and in some cases rats, for times varying from 10 seconds to 6 and 10 minutes. Application of 47°C up to 6 minutes produced no visible change. At 50° to 55°C, applied for 1 minute and over, there was a critical temperature for the development of permanent and irreversible damage, in animals good scab formation occurred after burning at this temperature. After temperatures of 60° to 65°C the epidermis could be peeled off from the exposed area, leaving a punched-out exposed surface area somewhat like the exposed human blister. A temperature of 70° to 80°C for 10 to 20 seconds produced severe scabbing. Observations of edema formation were checked by wet and dry weights of specimens of skin taken 2 hours after heating. At 55°C there was some change, but this was more definite at 60°C. Two different types of histologic reaction were described. With milder burns there was cellular disintegration, with more intense burns, heat coagulation. In more intense burns there was a peripheral shell of changes characteristic of burns of lower temperatures. Two substances were apparently lost by the epithelium of the burned skin, namely (a) basophil granules from the cytoplasm, and (b) nucleoprotein from the nuclei. These two substances could be identified in the intercellular blister spaces. Finally Leach and his associates found that collagen fibers change in structure and staining affinity in the more intense burns.

Prinzmetal and his associates in Los Angeles also reported experiments indicating different effects from mild and severe burns.

1 Rats which were mildly burned at 75°C for ten seconds on a single hind limb developed marked visible edema with considerable local fluid loss but did not develop shock.

2 Rats whose single hind limbs were severely burned at 100°C for from two to three minutes died in shock without visible edema and exhibited insufficient local fluid loss to account for death. Similar results were obtained when larger areas of the body were severely burned for ten seconds.

These authors concluded "It is therefore apparent that there are at least two mechanisms capable of producing shock, one due to local fluid loss and the other due to some unknown factor(s) "

Ham (1944) reported that in more severe second degree burns the skin surface tended to be white rather than red. Microscopically this was seen to be due to the superficial set of vessels being coagulated. Plasma loss in this type of burn originated from deeper capillaries. In this connection, Ham pointed out that the substance of the skin of both man and the hog is not evenly supplied with capillaries. He found that, in burns severe enough to seal off the superficial set, the next important set to become involved was that associated with the

deepest portions of the hair follicles and the deep secreting portions of sweat glands. These become dilated and congested and leak plasma. The leaking plasma, moreover, escaped from the skin down into the subcutaneous tissue. In more severe burns a third great capillary bed was affected, that of the subcutaneous tissue.

The possibility is to be considered that necrosis may result from trauma or treatment received after the original burn. Pressure dressings as a method of local therapy protect the burned area from mechanical or septic trauma. On the other hand, it has been shown by Taylor (1936), Cannon and Cope (1943), Hirshfeld, Pilling and Maun (1943) (on men), Maun, Schneider, Pilling and Hirshfeld (1943) (on dogs), and Clowes, Lund, and Levensen (1943) that other local remedies, tannic acid in particular, may increase the already existing necrosis of the skin and destroy remaining skin islands. Clowes, Lund and Levensen (1943) studied 150 cases of burns from the point of view of comparing results following surface applications of ointment gauze dressings, tannic acid silver nitrate, and triple dye. Areas treated with vaselined gauze became free of slough and were grafted earlier than areas treated with the other two methods. Dmgwall and Andrus (1944) studied a controlled series of 82 second degree burns in human volunteers and included observations on the efficacy of 12 different local treatments. A sulfonamide-impregnated plastic film gave the best results, a bland ointment locally combined with sulfonamides by mouth gave the next best results, while "our experience in this series of experimental burns gives additional evidence for the abandonment of any type of treatment with escharotic agents."

3 *Edema*. All burns of second or third degree have accompanying edema. In fact the edema following thermal injuries is one of the most outstanding results of such trauma. Drinker (1944) stated in this regard "Abnormal leakage from the capillaries is the dominant continuing characteristic of the burn lesion." In human beings the loss is both into the tissues and from the burned surface in the form of weeping. The amount of edema is considerable and was shown experimentally by Blalock (1931) to be sufficient to cause shock (i.e., 3.34 per cent of the body weight). The nature of the fluid lost is also of importance. Beard and Blalock (1931) (tissue edema fluid) and McIver (1933) (blister fluid) and others demonstrated that the exudate is plasma like and not just a watery transudate. Pressman, Janota, Weston, Levinson, and Necheles (1943) made simultaneous analyses of blister fluid and blood plasma. Blister fluid contained a concentration of proteins equivalent to 70 to 80 per cent of the plasma proteins. The albumin content of blister fluid was fairly constant, but the globulin content showed decided variations independent of the albumin or globulin values of the blood and of the albumin values of the blister fluid. These results may be correlated with those of Lischer, Elman, and Davey (1944) who found that in experimental burns in dogs the serum albumin concentration tends to fall. In their experiments, the globulin fraction also fell in relatively less severe burns, but high levels were often noted in fatally burned animals or in those subjected to higher thermal stimuli. The rate of formation of the fluid



is rapid and it occurs largely during the first 18 hours and practically stops after the first 48 hours (See item 13 )

With the rapid formation of large amounts of plasma-like fluid, the therapeutic implications would seem to be clear. The use of pressure dressings, of plaster casts or of cold (vide infra) represents an external means of controlling loss of fluid, while the recent paper of Berman, Peterson, and Butler (1944) introduces an internal method of accomplishing the same thing. These authors injected isotonic solution of sodium chloride hypodermically into the burned area in experimental anesthetized animals. Such an injection increases the hydrostatic pressure within the tissues and apparently in this manner minimizes plasma leakage. As a result of this treatment the survival time was considerably increased in animals so treated.

The edema of burns is associated with local vascular injury. As to whether or not there is a generalized increased capillary permeability considerable discussion still exists. Fine and Seligman (1944) injected plasma proteins tagged with radioactive iodine into dogs with burn shock. No evidence of leakage due to a change in the permeability of the generalized capillary bed was found. Tagged plasma proteins escaped into the area of injury in large amounts, but not into untraumatized areas. Cope and Moore (1944) studied capillary permeability in experimental burns using radioactive dyes. Following a hot water burn of a leg, the concentration of radioactive colloids in the lymph rose abruptly, indicating an increased capillary permeability to these substances. In only one experiment was there evidence of increased capillary permeability in an area remote from the burn. This single exception was an instance of late shock.

Netsky and Leiter (1943) used an immunologic method to study local and general capillary permeability in burn shock. The presence of horse serum after intravenous injection was tested turbidimetrically in the lymph. It was found that there was a prompt increase in the passage of protein, both in the burned and non-burned areas. This experiment thus gives results at variance with those listed in the preceding paragraph where the radioactive ion method was utilized.

Studies of the mesenteric vessels by the method of Knisely (1943) as used in burned cats by Abell and Page (1943) offers promise in the elucidation of the mechanism of burn edema and plasma loss. The work of Menkin (1943 and 1944) is also apropos. This author has isolated a substance from inflammatory exudates which shows irritating properties and causes leukopenia on injection. It does not depress the blood pressure of the cat. Menkin named this substance necrosin. It is antigenic and can induce a high precipitin antibody titer when injected into rabbits. An additional substance, leukotoxine, acts differently than necrosin in that it is leukocytosis-promoting, and is non-antigenic.

Evans and Hoover (1943) pointed out that in second degree burns most of the plasma loss is to the exterior in the form of weeping while in third degree burns it is chiefly into the tissues. "In our experience, there is practically no external loss of plasma in 3rd degree burns, the loss there is the 'white hemorrhage' of Allen and Koch, plasma lost into the subcutaneous tissues under the burn

It is in the 3rd degree burn that one sees the poorest results when tannic acid is used. To us it seems wholly illogical to cover the surface of a 3rd degree burn with a heavy eschar to prevent plasma loss. The net result is no decrease in plasma loss." Several unsolved problems exist in connection with the edema of burns. One considers the fate of the fluid and is discussed below under item 5. The other relates to the possible toxicity of the fluid and will be discussed in connection with item 16. Ham (1944) also studied the edema in burns of different depths and its relation to the tannic acid method of treatment.

Hirshfeld, Williams, Abbott, Heller and Pilling (1944) point out that "3rd degree burns do not 'weep' significantly during the first 48 hours because the burned skin has so coagulated that it forms a firm leathery insensitive eschar. However, after a few days this eschar softens and begins to slough, allowing copious exudation which persists until skin grafting has been completed."

4 *Compression treatment.* In 1924, Blair of St. Louis stated "There are chiefly four basic things to be gained by the use of properly applied mechanical pressure to wounds. The elimination of dead spaces. The control of oozing. The limitation of venous and lymph stasis. Limitation of the amount of plastic material that pours into the wound." The method of pressure dressing treatment of wounds particularly used by plastic surgeons Blair, Davis, and others, was applied to burns by Koch of Chicago (1941) and soon adopted by others. Allen and Koch (1942), Siler and Reid (1942), Cope (1943), Evans and Hoover (1943), Koch (1943), Owens (1943), Harkins (1943), Gurd and Gerrie (1944), etc. Excellent reviews of this aspect of burns are given by the National Research Council (1943) and by Lund (1943). This method involves a compression dressing which is changed infrequently. Koch (1944) stated "The essential features of such treatment are surgical cleanliness, compression of the injured surface to prevent fluid loss, and rest." Another means of giving uniform pressure without disturbing the wound is the use of rigid "theroseal" jackets as advised by Douglas (1944) by means of which hydrostatic pressure is applied.

The work of Burch and Winsor (1944) on the rôle of the skin and corneal layer in edema formation is somewhat apropos to this discussion. These authors showed that the dead, keratinous, even desquamating, thin, corneum acts as an elastic "pressure dressing" whenever edema forms. Possibly one of the causes of burn edema is that due to the thermal trauma this natural defensive mechanism breaks down. Roback and Ivy (1944) concluded from their experiments on dogs that "a firmly applied dressing decreases the time required for the complete epithelization of a burn wound."

Modern pressure dressings are usually of 4 layers: fine mesh gauze next to skin, surgical squares, loose fluff (mechanic's waste), and a compression roller. The nature of the first layer is the chief focus of debate. Everything from dry gauze or rayon to boric acid ointment (Cope, 1943) or sulfonamide permeated gauze is used (Harkins, 1943). Farr (1944) modified the inner layer of the occlusive dressing of burns by applying sulfanilamide ointment to the surface and then in turn covering this with transparent cellophane. Pressure was

applied to this and the method was used in 10 second degree burns with success. Hawn, Bering, Bailey, and Armstrong (1944) presented observations of surgically denuded areas of animals and of burns on humans on which fibrinogen and thrombin were used as a coating. No ill effects were noted and such films acted as a satisfactory dressing. The fibrinogen and thrombin were obtained as by-products of the processing of blood plasma at Harvard Medical School. The authors suggested that "such films, particularly in the form of roll bandages, might prove a highly expedient fibrinogen-thrombin dressing for burns in the field, owing to simplicity and speed from the standpoint of application, and to lack of bulk from the standpoint of transportation." This mode of treatment is somewhat similar to the use of human fibrin by Macfarlane (1943) in eight cases of accidental burns and two cases of experimental human burns.

The recent observations of Pfeiffer, Hallman, and Gersh (1944) on the toxicity of boric acid ointment used in the treatment of burns are of especial interest. These authors tested the ointment on dogs and found that "treatment of a burn involving only 4 per cent of the surface area of the body with U S P 10 per cent [boric acid] ointment, will produce pathological changes in the central nervous system." These authors concluded as a result of their work "Boric acid, whether applied in the form of an ointment or saturated solution to extensive wounds is a cumulative poison. The weak antiseptic value of boric acid suggests that for most uses, other more active and less potentially harmful therapeutic agents should be employed."

This work on the toxicity of boric acid repeats an old story in the history of burn therapy, one noted before in the case of picric acid and later of tannic acid treatment. This is that each time man attempts to become more elaborate and use anything other than the simplest of coverings on an extensive burned surface, he is apt to do more harm than good.

Another means of exerting pressure is to apply a rigid dressing before swelling has taken place and to allow the edema to exert its own pressure. This is the basis for the use of plaster casts as advocated by Glenn, Gilbert and Drinker (1943) (experimental), Levenson and Lund (1943) (clinical), Lund (1943), Sellers and Willard (1943), Barnes (1943), Sellers and Goranson (1944), and Glenn (1944) reported the use of plaster casts in 16 cases of infected burns of the extremities with good results. Most of these cases were treated in from 4 to 21 days after injury, only one being seen sooner (17 hrs). Thus, this report is concerned essentially with a different problem than the primary treatment of burns. Elevation of the burned part, especially when plaster is used, was emphasized by Barnes (1943) as a means of reducing edema. Another physical method of reducing edema and plasma loss is the application of local cooling as done by Sellers and Willard (1943) and by Allen (1944). The principles which govern the treatment of burns with plaster casts as outlined by Barnes (Great Britain) (1943) are "A simple technique of treatment, which includes the use of Plaster-of-Paris casts for burns of the hands and forearm, is described. If plaster is applied, elevation of the limb is essential, to prevent edema, and the hand must be placed in the correct 'position of function'." The value of immo-

bilization, infrequent dressing, and the prevention of edema is pointed out. Burnt hands do not become stiff merely as a result of immobilization, provided that this is carried out correctly for a limited period. In spite of the presence of infection with *Ps. pyocyanea*, skin grafts will 'take' on granulating burns that are dressed infrequently."

Glenn (United States) (1944) stated these same principles as follows: "At the present time these directions for using closed plaster dressings in the treatment of burns may be followed. After minimal cleaning and debridement and without breaking blisters, two layers of sterile gauze are laid over the burned surface extending over the area to be covered by plaster. If fingers are involved they are enclosed separately in the gauze. A thin sheet of roller plaster bandage is then applied to the part without pressure, extending over the end of the extremity and two to four inches above the burn. In mild burns the encasement is removed in 7 to 14 days. In more severe burns it may be left in place for as long as four weeks. If the burn is incompletely healed or the epithelium is very thin, another encasement should be immediately reapplied. Grafting may be started as soon as the slough separates."

Lischer and Elman (1943) reported that in dogs the use of local elastic pressure after burns decreased the degree of hemoconcentration, but did not reduce the mortality. The importance of tissue pressure in the edema of burns was demonstrated by Bellis (1942) and Rossiter (1943). This may increase from 1-3 mm Hg to 10-20 mm Hg. Rossiter showed that pressure of the order of 10 mm Hg applied externally will gradually lessen the edema in the skin of a burned guinea pig or rabbit. Another method of increasing tissue pressure is to inject saline solution into the burned area as was done by Berman, Peterson, and Butler (1944).

One of the purposes of the tannic acid treatment of burns is to prevent plasma loss. It manifestly does stop weeping from the burned surfaces, but its action on tissue edema is less certain. Ham (1944b) stated that his work "provides no evidence to suggest that the tannic acid treatment would be equally efficacious in the more severe second degree burns or in third degree burns where plasma loss occurs primarily from capillary beds out of reach of the tan."

**5 Increased lymphatic flow** Lassar (1877) performed experiments in which a hind leg of animals was placed in water at 50 to 54°C for 5 to 6 minutes with resultant marked swelling. He reported that there was an increased flow of lymph in scalded legs and believed that this was the mechanism whereby the fluid was reabsorbed and a return to normal effected. Wood (1940) tested lymph collected from the thoracic ducts of burned animals for vasodepressor activity by intra arterial injection into albino rats and found no evidence for such activity.

Glenn, Peterson, and Drinker (1943) extended previous observations of Field, Drinker and White (1932) on the lymphatic flow from a scalded hind limb of anesthetized dogs. They found a significant increase in lymphatic flow following the infliction of thermal trauma. Subcutaneous injection of tissue extract in the region of the burn caused a coagulation of lymph, with stoppage of lymphatic

flow Sections of such an injected burned foot showed definite fibrin formation while the non-injected burned foot showed practically no fibrin In addition, a burned foot with injection of tissue extract tended to swell much more than a non-injected foot because "local swelling depends notably upon the degree to which coagulation of the exudate occurs with imprisonment of the water which leaks from the blood capillaries" Further evidence for this hypothesis is afforded by the observation that heparinization of the dogs to the extent that formation of fibrin was prevented caused a minimal swelling of the foot after thermal trauma Glenn, Muus, and Drinker (1943) studied the lymphatic flow from a scalded leg of a calf and compared its amount and chemical composition with that from the opposite untraumatized limb The results were interpreted on the basis of the following logical assumptions "We assumed that lymph coming from the burned area would contain a higher concentration of substances resulting from tissue damage due to the burn than would lymph from another part of the body, or serum taken from the general circulation On the other hand, substances of small molecular size which accumulate in the serum owing to impaired function of any of the organs would be present in the same concentration in the serum and in the lymph collected from different parts of the animal "

Their studies seemed to indicate no increase in capillary permeability in normal regions of the body distant from the burn They concluded that the increase in urea and creatinine was consistent with the usual observations attending oliguria In their opinion the increase in creatine was due to local damage, while the increase in amino acid was of uncertain origin This observation concerning rise in amino acid in shock due to burn fits in with the observations of Harkins, Long and associates (1944) The rise in nonprotein nitrogen was just as great in the serum or lymph from an unburned leg as in the lymph from the burned leg and hence does not seem to arise primarily from the region of local trauma

Perlmann, Glenn, and Kaufman (1943) described a new globulin fraction in lymph collected directly from the burned area in calves Since lymphatic flow from the site of thermal trauma may act in two ways to hasten local repair or to carry off possible noxious products, this globulin may represent a "toxin" but as yet no exact significance can be attached to this finding

6 *Infection* This remains one of the chief factors in burn mortality which resists all attempts at adequate prevention and treatment Graham (1944) lists the control of infection as *the* unsolved problem of burns Local application of sulfonamides, in powder form, at least, has been discarded Tennison (1943) reported that sulfasuxidine could be locally applied without rapid systemic absorption, but Poth and Ross (1944) refute this statement with a large series of clinical studies Oral sulfonamides are most generally used, while penicillin by various routes offers promises but has as yet not fulfilled all of them One fact of practical importance remains, namely, that symbiotic growth of skin and respiratory tract organisms is far more serious than are the former alone on a burned surface This is the rationale of masking of both the operator and, if

possible, the patient during major burn dressings and is an additional argument for the infrequent dressing of early burn wounds

While local use of powdered sulfonamides is not popular at present, interest has arisen in attempts to control burn infection by sulfonamide ointments (Allen, Owens, Evans, and Dragstedt, 1942, Jenkins, Allen, Owens, Schafer, and Dragstedt, 1945, and Gurd, Ackman, Gerrie, and Pritchard, 1942), of sulfonamide sprays (Coloviras, West, and Armour, 1942) or of sulfonamide films or membranes (Pickrell, 1942, Clark, Strakosch, and Leven, 1942, Skinner and Waud, 1943, and Andrus, Nickel, and Schmelkes, 1943)

*7 Rationale of early cleansing* As discussed under "compression treatment" above, Allen and Koch (1941), Siler and Reid (1942) and most advocates of this method recommended early cleansing of the burn wound with soft soap, water, and, occasionally, with fat solvents. At the time of the Coconut Grove disaster in 1942, on the recommendation of Cope, those burns treated at the Massachusetts General Hospital received all the elements of a good compression dressing except the usual preliminary cleansing. The results were good and this institution as well as several others have continued to omit such ablutions of the wound, but are extremely careful not to introduce additional infection including that from the respiratory tract. Of the three kinds of debridement of burns—soft cloths, harsh scrubbing brush, and scalpel excision with immediate skin grafting, the first is the one under discussion here. The second accomplishes too little to compensate for the added trauma and necessity for general anesthesia it entails. The third offers promise in selected cases, but their number will always remain small. Furthermore, because of lack of early diagnosis of burn depth, the embarrassing possibility of excising and skin grafting a second degree burn is always to be considered.

The no-debridement method has the following rationale according to Cannon (1944): "No debridement of the burned surfaces was done because evidence accumulated indicated that an increase in contamination of the wound resulted. Only by vigorous scrubbing of the surface could this contamination be reduced. However, the trauma of such scrubbing necessarily causes damage to viable cells and reduces the effectiveness of the important factor of tissue resistance in preventing invasive infection." A fine mesh gauze is placed on the burn without rupturing the blisters. This plan is justified by the absence of bacterial growth in cultures of blister fluid taken at intervals after the burn. The outer wall of intact, or even of ruptured blisters, acts as a protective membrane and may be considered as Nature's dressing for the ideal covering for a burn. Lund (1944) epitomizes this as follows: "The less often you dress a burn the faster it heals," and also states: "I have become such a therapeutic nihilist on the subject of chemical applications to the surface of burns, that as some people know, we have given up any applications now except dry gauze, counting on rest, pressure with or without plaster, as creating as good conditions as we know of for the healing of a burn, and allowing those dressings to remain up to three and even four weeks."

*8 Determination of the area and depth of the burn* The methods of Weidenfeld

(1902) and of Berkow (1924) form the basis for the calculation of the area involved by a burn. By breaking the body surface up into segments, this estimation becomes easier and the Berkow method is the standard used in hospitals throughout the world. Lund and Browder (1944) have adapted this method to patients of all ages, showing that the surfaces of three crucial areas of the body (head, thigh, and leg) can be considered to vary relatively with age, whereas the other areas may be considered to be of constant proportions. The importance of the proportionate surface area burned is that it may serve as a rough guide to treatment, especially as to the plasma needs during the first 24 hours (Harkins, 1943).

The depth of the burn is usually classified according to the listing of Boyer or Morton. First degree, redness, second degree, blistering, and third degree, eschar formation. A more practical classification is into superficial and deep, or into those which do not and those which do granulate. The classification of Converse and Robb-Smith (1944) (see table 1, below) is of interest in this regard. Since granulating burns of any considerable size always need skin grafting, the importance of this differentiation is manifest. Unfortunately, it is extremely difficult even for those with considerable experience to tell how deep a burn is when it is first seen. A favorable result following an early incorrect diagnosis of third degree burn is the basis for the empiric adoption of countless local burn remedies.

Dingwall (1943) observed burns after sodium fluorescein injection with ultraviolet light and a filter. This author found that third degree burns were blue-black, whereas second degree injuries appeared yellow-green, generally more intense in color than the surrounding skin. This method was tested successfully on third degree burns in experimental animals and on both second and third degree burns of patients. Patey and Scarff (1944) took sections from clinical burns and also from experimental burns of the skin of breasts produced immediately before removal for carcinoma. A standard technic for producing experimental burns in human skin was described. Histological examination of the shed blistered skin was found to be of value in the determination of the depth of damage and in the control of treatment. Such examination was especially valuable if it showed the plane of separation to be intraepithelial, it was of less value when it showed complete epithelial separation, as there was then no indication of the presence or absence of the underlying damage. The degree of dermal destruction could be assessed by differential staining of whole-thickness skin sections, but the taking of such sections on a large scale has practical objections. Finally, Patey and Scarff found that a modified Van Gieson's stain, when applied to the burned area of patients gave some useful naked-eye indications of the degree of dermal involvement.

9 *Separation of burn sloughs* Once the acute phase of a third degree burn is over, an inactive period of waiting several weeks for the burn slough to separate is always necessary before skin grafting can be done. Such a wait seems useless, but on the other hand may be Nature's best method of handling the situation. At least skin grafting cannot be done on top of dead tissue. Attempts to hasten

the separation of the slough have been made by Connor and Harvey (1944). These authors have used pyruvic acid at a pH of 1.9 in 8 per cent cornstarch and found that the slough of experimental burns could be removed in 48 to 72 hours so that early skin grafting could be employed at the end of that period. Cooper, Hodge, and Beard (1943) had previously used an enzymatic digest for the removal of dead tissue following burns. Howes (1943) noted that such digestion damaged living tissue in some instances, whereas Connor and Harvey (1944) stated that with a carefully adjusted pH, early slough removal 'can be accomplished without significant injury to viable tissues'.

These methods are reminiscent of the acetic acid method of burn treatment introduced by Hartwell (1917) and popularized by Dorrance and Bransfield (1922). This technic was considered to be a method of "chemical debridement."

**10 Healing and regeneration.** Glenn (1944) pointed out that there are four chief factors which interfere with burn healing: (1) too active cleansing or scrubbing of the part at the time of initial debridement, (2) the application of harmful local therapy, (3) infection, and (4) inordinate swelling of the subcutaneous tissue with resultant stretching of the skin and anoxia of its cells, especially in poorly vascularized areas or in regions where the skin is very thin. As already stated under the discussion of "necrosis," certain local applications to burned areas may actually retard healing and regeneration following such injuries. Other applications are made to burned surfaces late in the course of the lesion to hasten epithelial regeneration. The work of Brush and Lam (1942) is a classic in this field and demonstrates that most such agents do more harm than good to wounds of non thermal origin. Auerbach and Doljanski (1944) applied a saline extract of adult chicken heart and an alcoholic precipitate of this extract, as well as a saline extract of seven days old chicken embryos to experimental skin wounds in rats. No increase in healing time was noted as compared to control animals. Hamilton (1944) studied chemical burns and wounds and wounds in rabbits when treated with nickel pectinate using bland agents chiefly cod liver oil and foile as controls. This author found that nickel pectinate reduced the healing time 22 per cent, but did so by keeping down granulations rather than by stimulating epithelization. Smith and Livingston (1943, 1945) studied the effect of 17 agents on experimentally induced wounds and burns (1372 injuries and 878 controls). "Of all these agents, only the chlorophyll preparations consistently showed any statistically significant effect in accelerating the healing of both traumatic and thermal wounds." Williams and Bissell (1944) tested 11 substances on uniform-sized wounds of rats. These agents (nickel pectinate and chlorophyll were not included) had little effect and the authors concluded 'No definite benefit was derived from the use of any of these substances.' Cope (1944) summarized his opinion on this subject by stating 'No substance is known which expedites the healing of the wound above that obtained under conditions of normal nutrition and absence of infection.' The most recent mathematical studies of the rate of wound healing are those of Apperly (1944). Barclay, Cuthbertson, and Isaacs (1943) approached the subject of wound healing differently. Dried thyroid gland or



small doses of 2-4- $\alpha$  dinitrophenol, when fed to young adult male albino rats throughout the period of healing of circular skin wounds, caused a significant reduction in the near time of healing (11 per cent and 15 to 27 per cent respectively) These authors did not advise these drugs for clinical use

*Normal healing and regeneration is complicated by the constant presence of dead tissue plus infection* Interesting recent work is that of Fell and Danielli (1943) Small shallow burns were produced on rats anesthetized with ether with a soldering iron 1.5 mm in diameter applied for about one second Changes in the skin phosphomonoesterase were studied during the healing stage Pooled scabs were analyzed and the phosphatase content was estimated in terms of milligrams of chemically determined phosphate split off in a standard time In the shallow burns such values were especially high during the stage of regeneration (around the fifth day) Patey and Scarff (1944) studied healing after experimental burns They conclude There is no evidence for regeneration from the interpapillary processes of the skin (rete pegs) and from our observations it seems almost certain that the areas of regeneration formerly attributed to this cause are in reality derived from the ducts of follicles or glands "

Converse and Robb-Smith (1944) studied 469 skin graft donor sites and drew analogies between them and burns with respect to healing They found that the quality of repair was roughly proportional to the rapidity of healing During two years' service at the American Hospital in Britain (1940-1942) 191 burns were observed The healing of these burn wounds was observed and 63 biopsy specimens were removed for histologic examination As a result of these studies an anatomic terminology for classifying the depth of burns was used by these authors as shown in table 1 In deep burns where the full-thickness of skin has not been lost, healing occurs with considerable skin contraction and an invisible loss in skin surface is noted This contraction is called "inter-island contraction" by Converse and Robb-Smith In such cases, epithelial resurfacing originates from the individual epithelial islands formed from each hair follicle, sweat duct or cluster of sweat glands Each epithelial island tries to join its neighbors not only by cellular division and migration but also by contraction These authors believe it is logical to state that the longer epithelial healing takes to resurface a wound the more marked this inter-island contraction will be In such cases a thick layer of relatively avascular inelastic fibrous tissue is laid down which is covered by an atrophic epithelium of poorer quality

Converse and Robb-Smith believe that "inter-island-contraction" is more apt to occur when the skin of the area is loosely attached to the underlying structures, for example on the dorsum of the hand or on the eye-lids, the latter instance resulting in ectropion, or in the vicinity of joints where the contraction tends to interfere with normal motion These authors further state that the newly healed smooth shiny tight skin with avascular and inelastic scar tissue is the enemy of serviceable repair Palliative measures such as massage, ointments, and other physical methods have their place, but too much time may be wasted in using them, particularly in burns of the hands, and in most cases the best treatment is the complete replacement of such scars by thick skin grafts

These authors state that skin grafting is indicated in superficial burns for two main reasons (1) for the relief of skin deficiency of tightness, following inter island contraction of deep thermal and mixed burns, particularly on the dorsum of the hand, around joints, for ectropion of the lids or distortion of the features of the face, (2) to replace skin of poor quality Thin, shiny or keloid skin is poorly resistant to every day trauma It tends to crack and ulcerate, even as a result of cold weather Return of sensation is poor and the appearance of the skin is often disfiguring

II *The General Disturbances of the Burned Patient* From the standpoint of saving lives, these general effects of the thermal injury are of possibly greater importance than is the local burn lesion The correlation between the two is of great importance and should always be considered Reviews of this aspect of the subject are given by Lund (1943), Cope (1944), Lam (1944), and Harkins

TABLE 1  
*Classification of burns (Converse and Robb-Smith, 1944)*

Superficial burns (partial skin loss)	Epidermal burns Erythema Epithelial desquamation Dermal burns Blistering Destruction of superficial layers of the dermis	Heal well
	Deep dermal burns Destruction of the dermis down to the deep layers Mixed burns Small areas of total skin loss alternate with areas of deep dermal burns	Heal slowly with contraction may require skin grafting
Deep burns (total skin loss)	Destruction of whole thickness of the skin into or beyond the fat	Heal with difficulty producing contractions and deformities Skin grafting the rule

(1943, 1944) Several pertinent problems in the management of the general disturbances of the burned patient will now be considered in order as follows

11 *Morphine and anesthesia* Before the war, most burns treated in Great Britain were anesthetized before applying local therapy, while in this country general anesthesia was used only rarely At present, such general anesthesia is used even less often than before Morphine is used as an analgesic and such preliminary cleansing which cannot be done without supplementing morphine is considered too traumatizing to be advisable The only question which arises is as to how much and by what route the morphine should be given

Beecher (1943, 1944) pointed out that burn patients are apt to be hyperactive or even manic from three causes Pain, fear and hysteria, and cerebral anoxia Morphine is a useful therapeutic agent only for those in the first of these three groups In the other two groups it is not only ineffective but is contraindicated in large doses When morphine is given subcutaneously to patients in shock it is apt to remain unabsorbed until shock therapy has been administered After restoration of blood flow several doses of morphine might be absorbed

at once, with resultant collapse. Beecher points out that morphine should be administered intravenously to such patients. If because of the large number of cases to be cared for, one cannot take time for intravenous administration of morphine, the agent should be injected into an unburned extremity and the injection site marked with ink or dye so that if too great absorption results later on the inflow can be checked by means of a tourniquet.

Elman (1944) found in experimental burns that morphine increases the 24-hour mortality by as much as 100 per cent, particularly when given in large doses and with nembutal. He stated "It is inferred that large doses of morphine when used in the absence of pain may increase the early mortality in severe burns."

12 *Burn shock*. Burn shock is chiefly a menace during the first 24 to 48 hours after the burn and is responsible for about two-thirds of burn deaths. While other mechanisms may play a rôle, the oligemia which results from plasma loss (item 13) is one of the chief factors. As a result of the oligemia, various cardiodynamic factors occur which are not primarily due to cardiac or vascular failure. It is true that cardiac damage does occur in burns (item 16), but essentially the heart and blood vessels are relatively unaffected, while the amount of blood they have to deal with is seriously depleted. Elman and Lischer (1944) summarized the importance of this latter factor by stating "The production of inflammatory edema alone is clearly the cause of loss of plasma leading to hemoconcentration, fall of blood volume, and the physical changes responsible for surgical shock." The cardio-dynamic factors in burns include decreased cardiac output, diminished blood flow, peripheral vasoconstriction, intact vasomotor center, decreased venous pressure, and a late fall in arterial pressure. These items are all interrelated and in turn are all essentially secondary to the oligemia. Abell and Page (1943) studied the smaller blood vessels in anesthetized burned dogs and cats by direct vision. Vasoconstriction of the larger and smaller arteries and larger veins and constriction of arteriovenous anastomoses were noted.

It is somewhat uncertain as to whether a nervous factor exists in shock due to burns. Kabat and Hedin (1942) presented data of interest in this regard. Experiments were carried out on anesthetized cats, some of which had the spinal cord sectioned at  $L_1$  or  $T_{11}$ . No appreciable difference was noted in the survival time of the two groups. The animals with the spinal cords sectioned showed much less hemoconcentration and local edema at the site of the burn than the control burned animals. These experiments seem to show that animals with transected cords behave differently after burns than do previously intact animals. They demonstrate that there is a nervous factor in burn shock, not that there is one in the causation of burn shock.

Adrenal cortical extract has been utilized in the treatment of burns. Rhoads, Wolff and Lee (1941) reported promising results from the use of this substance. They estimated that after a severe burn the capillaries do not regain their normal permeability until the fortieth hour, but after treatment with adrenal cortical extract such normality was attained by the eighteenth hour. A more recent report based on additional cases by Rhoads, Wolff, Saltonstall and Lee (1943)

reverses this opinion stating that "the results of this larger series have not fulfilled the promise of the earlier cases. They do not provide a satisfactory basis for the use of adrenal cortical extract in the routine treatment of shock following burns." These clinical observations are corroborated by the experimental studies of Rosenthal (1943) who tested standardized burns in mice and found no protective action whatever by either desoxy corticosterone or adrenal cortical extract injected subcutaneously as compared with controls with saline solution.

**13 Plasma loss and plasma therapy** Following a severe burn, various hematologic changes ensue. The early hemoconcentration following burns first noted by Baraduc (1862) is so well known as to need no further comment. With the marked loss of plasma and resultant relative cell increase, there is a notable oligemia, possibly the most important of all the general changes in burns. There is apt to be a relative decrease in albumin and chloride, and a rise in potassium and other evidences of cell disintegration. The sedimentation rate is slowed early and increased later. The white blood count is greatly increased in the early stages and remains elevated if infection supervenes. The platelets may decrease markedly in number (MacDonald, Levenson, Davidson, Tagnon, and Taylor, 1944).

The possibility that the local edema and plasma loss could account for the hemoconcentration and burn shock was first postulated by Tappeiner (1881) and demonstrated quantitatively to be sufficient by Blalock (1931). The recent reviews of Blalock (1940) and of Rossiter (1943) are to be consulted in this connection. Rossiter concluded "It is, therefore probable that these enormous losses of plasma from the blood-stream will initiate shock. Even should this prove but one of a number of initiating factors, it is one of immense importance and one which justifies vigorous counter measures."

These counter measures include especially, transfusion of plasma, albumin or whole blood of an amount calculated to fit the needs of the individual patient. The details of the treatment of burn shock were reviewed by Harkins, Lam, and Romence (1942), McClure and Lam (1943), and by Lundy, Adams and Seldon (1944). The use of gelatin in experimental burn shock was reported by Parkins, Koop, Riegel, Vars, and Lockwood (1943). Seven of the eight treated dogs died.

The need for plasma therapy has been recognized since Blalock's demonstration in 1931 that in fatal burns sufficient plasma is lost to produce death. Rossiter (1943) stated "Shock is one of the most obvious clinical manifestations of severe burns. Evidence is given for the view that the plasma loss known to occur after severe burning is in most cases sufficient to initiate shock." Elman (1943) presents statistical data demonstrating the value of plasma transfusions. The time when such transfusions are essential is variously stated as the first 36 hours (Rhoads, Wolff, and Lee 1941) and by inference from the hemoconcentration curves of Elman and Brown (1943), the first 14 hours. Few persons with burns of less than 10 per cent of the body surface burned will require plasma transfusions. Lombard (1943) reported on the use of plasma transfusions following burns as used in Africa.

Plasma dosage is estimated in three ways extent of hemoconcentration, area of the burn, and clinical judgment

If laboratory facilities are available, the following methods of estimating plasma dosage based on blood concentration may be used

- 1 Formula of Black (1940)
- 2 Formula of Elkinton, Wolff, and Lee (1940)
- 3 More recent formulas (of author, 1941-1944 except where stated otherwise)
  - (a) Give 100 cc of plasma for every point the hematocrit exceeds the normal of 45,
  - (b) Give 50 cc of plasma for every point the hemoglobin exceeds the normal of 100,
  - (c) Give 300 cc of plasma for every gram the hemoglobin exceeds the normal of 15 grams per 100 cc (Phillips, 1944),
  - (d) Give 100 cc of plasma for every 100,000 the red cell count exceeds the normal of 5,000,000 per mm<sup>3</sup>,
  - (e) Give 150 cc of plasma for every specific gravity increase of 0.001 above the normal whole blood specific gravity of 1.060 (Phillips, 1944)
- 4 Nomogram of Jenkins, Schafer, and Owens (1943)

In children the amount of plasma to be given should be proportionately less according to body weight. Furthermore, the calculations do not indicate the amount of plasma that will be needed to manage the entire course of the burn. Just as in a diabetic patient when the blood sugar indicates that 30 units of insulin is necessary, this does not mean that 30 units will suffice for a whole month, so in a serious burn case frequent determinations are essential. Hematocrit readings should be taken as often as every three hours during the first day after a severe burn and the amount of plasma indicated each time given. The danger of burn shock is not over until the thirty-sixth to the forty-eighth hour.

Administration of plasma in proportion to the burned area is of value where laboratory facilities are available. Use of "first aid formula" (1942) involves the administration of 50 cc of plasma for every per cent of the body surface burned by a deep (blistering) burn (see item 8). This amount should not be administered all at once, but according to the following schedule: one-third the first two hours, one-third the next four hours, and one-third the next six hours. Presman, Janota, Weston, Levinson, and Necheles (1943) do not believe that this formula gives quite enough plasma and suggest the following schedule. In the treatment of extensive burns in adults the immediate administration of 50 cc of serum (or 60 cc of plasma) for every per cent of body surface burned, and, in addition, 20 to 30 cc for every per cent should be administered during the first twenty-four hours and another 10 to 30 cc in the first seventy-two hours following the burn. Total administration, therefore, should be at least 100 to 110 cc for each per cent of body burn. Others believe that 100 cc for each per cent of the surface burned is too large a dose. In general, burns of the face, groin, or buttocks usually lose more plasma than the surface involvement indicates and more plasma should be given accordingly.

14 *Burn shock complicated by pulmonary damage* The importance of pul-

monary damage in burns was first fully recognized by Wilson (1942). The incidence of such lesions was very high in the Coconut Grove disaster, and the resultant studies at the Massachusetts General Hospital led to the excellent group of articles by Beecher (1943), Aub, Pittman, and Brues (1943), Schatzki (1943), Mallory and Brickley (1943), and Cope and Rhinelander (1943).

Pneumonia may be of local or general causation or both. Thus, local damage due to inhalation of flames or hot air leads to pulmonary edema which may become secondarily infected. In certain instances pulmonary edema (see table 2) may also arise from a shocklike state. Walton (1944) stated in this regard "Tracheotomies in those patients who have inhaled fumes are essential and life saving. If there is any question as to the need for this operation, it should be carried out without delay." This same author emphasized the general importance of sepsis in burns, stating "Most of the patients with burns who die after a ten-day interval, die from sepsis." A summary of the present status of pulmonary edema in burns is given in table 2.

TABLE 2  
*Pulmonary edema in burns*

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*Etiology* Pulmonary edema usually occurs from (a) inhalation of flames or very hot air or steam, (b) inhalation of noxious gases released at the time of the burn (e.g., Coconut Grove disaster), and (c) more rarely as a part of generalized increase in capillary permeability or when there has been overtreatment with parenteral crystalloid solutions.

*Time of onset* In fulminating cases after several hours occasionally after two or three days.

*Treatment*

1. Avoid excess fluids
  2. If coincident shock requires therapy whole blood is best and plasma next best, while crystalloids may be dangerous
  3. Turn patient frequently
  4. Pressure breathing of oxygen
  5. Early tracheotomy in selected cases
- 

15 *Importance of environmental temperature in influencing the mortality following severe burns* Before 1941, no advice concerning the treatment of burns was complete without mention of the so-called burn tent, usually kept at 85–110°F. In the light of present knowledge, such tents serve as perfect incubators for bacteria in the burn wound and markedly aggravate any shock which might be present. Since the report of Blalock (1941) concerning the dangers of excessive heat in shock, it is now recognized that all such tents are extremely harmful. Furthermore, with modern compression bandages, the patient is usually sufficiently insulated actually to benefit by a slight coolness of the surrounding temperature. This work has since been substantiated by Devine (1943) and others. Elman, Cox, Lischer, and Mueller (1942) studied the 24 hour mortality percentages of rats similarly burned by dipping into hot water at constant temperature of 80°C, but kept at four different temperatures afterwards. All animals placed in rooms at 32° and 99°F were dead within 24 hours. At 55°F, 82 per cent died, while the lowest mortality of 26 per cent occurred when the animals

were kept at 75°F This would seem, then, to be an optimum temperature, with increased mortality in colder or hotter environments, particularly the latter

16 *Burn toxemia* As Blalock noted in his recent monograph (1940) there is more evidence in favor of a toxic theory in burn shock than in most other forms The problems of toxemia in burns were discussed by McClure and Lam (1940) One point favoring the toxic theory is that the amount of shock present is not always sufficient to account for subsequent death In fact, many burn patients die who have never had shock at all Toxins afford a possible explanation for these deaths Such supposed toxins may act directly on various cells or organs or may affect them indirectly by means of accompanying metabolic or endocrine disturbances The relationship between the degree of skin damage and the possibility of toxemia has already been cited above in discussing the subject of necrosis (item 2) as an example of local pathologic change

Rose and Browne (1942) made studies on the blood histamine, hemoglobin, hematocrit and plasma proteins following severe burns in seven patients They found in certain cases a definite increase in blood histamine within one hour after the burn In all cases, however, there was a decrease in blood histamine with the appearance of toxemia and edema With the disappearance of these two noxious signs and with clinical improvement of the patient, the blood histamine rose to normal or higher levels In their patients, such secondary increases in the blood histamine persisted above the normal for some time in certain instances Rose and Browne noted a definite correlation between the degree of damage and the decrease in the histamine content of the blood Thus, the greatest decrease in the blood histamine was observed 12 to 36 hours before death These authors finally observed that the changes in the histamine content of the blood do not appear to be altered by the administration of plasma in amounts sufficient to control hemoconcentration

The observations of Perlmann, Glenn, and Kaufman (1943) have already been cited in which they described a new globulin fraction in lymph collected directly from a burned extremity in calves Antos, Dworkin, and Green (1944) studied shock associated with deep muscle burns Such burns were produced aseptically in the hind legs of anesthetized dogs by means of strong diathermy currents Five dogs so burned and restrained under anesthesia died with only moderate fluid loss as measured by a plethysmographic method Five other dogs similarly burned but not restrained after burning survived and when killed showed marked fluid loss These authors concluded that "The accumulation of fluid at the site of the burn is the principle factor responsible for the prodromal stage of shock in the burned dogs Association of the extensive muscle destruction with restriction of movement and with prolonged anesthesia converts the prodromal stage into outspoken shock with death of the dog even when the accumulation of fluid at the site of the burn is quite small "

Anuria is one feature of serious burns which may be either a cause or a result of toxemia Olson, Walker, and Necheles (1944) studied the urinary excretion in dogs burned under nembutal anesthesia Anuria promptly resulted which did not always respond to massive doses of saline by intravenous injection In

one experiment, despite a systolic blood pressure of 100 with anuria, a total of 2400 cc of saline had no effect on kidney secretion. "The difference in response might be attributed to different amounts of hemoglobin in the plasma. Permanent damage to the kidneys and anuria may develop following crushing or burning, because renal function seems to be most impaired following these two traumatic agents."

Miyata and Kayashima (1941) made clinical observations on patients with scalding burns. An early suppression of urine with gradual return to normal was noted. Urobilin and urobilinogen were demonstrated in the urine in about 50 per cent of the cases during the first week.

Kayashima (1940) reported electrocardiographic changes in patients and rabbits with burns. In 12 naval casualty cases (21-37 yrs) in which 1/5 to 1/8 of the body surface suffered burns electrocardiograms which were taken denoted that the Q and S waves were deeper, the R wave lower and the T wave flatter than their respective normal waves, while the P-Q and Q-T values were almost unchanged. Clinically the patients had very small pulse pressures, and one showed absence of the sino-auricular contraction and three dominance of the left ventricles. In about half of the patients the dysfunction of the cardiac muscles was clearly noted on the electrocardiogram. With the improvement of the lesions the muscles showed a gradual recovery of their normal functions but a certain amount of their functional impairment remained for a relatively long time. In further studies, Kayashima found that after experimental burns were inflicted on 10 rabbits the electrocardiograms showed changes in the waves which were characteristic of the impairment of the cardiac muscles, noted in the electrocardiograms of patients suffering from burns. All the waves were low in amplitude, the T wave was flattened out and the ventricular complex tended to be monophasic. In 3 cases the excitation conduction was interfered with, producing extrasystole, auricular flutter and absence of the sino-auricular excitation. Histopathologically interstitial myocarditis with perivascular infiltration of the leucocytes was observed in 5 cases out of 7 autopsied. These results of Kayashima (1940) are to be correlated with those of Simonart (1938) who also worked on rabbits. It is of interest that Kayashima found flattened T waves while in Simonart's experiments they were higher than normal. Histologic changes in the heart have been reported in human burn cases by Zinck (1938) and by Buis and Hartman (1941). In Simonart's experiments on rabbits, pathologic alterations in the heart included focal necrosis, calcification, some cellular infiltration and proliferation of connective tissue. Fain (1939) and Gunther (1939) also noted pathologic changes in the heart following burns.

The question of the causation of Curling's ulcers following burns still remains unanswered. One thing is certain, however, that they are less common now than they were a hundred years ago. In 1842, when Curling saw 4 personal cases at the London Hospital in the 19 month period beginning September 1840. In a review of the literature, Harkins (1938) decided that the infectious theory was the most likely and the recent report of Hartman (1945) agreed that infection is at least one of the three chief etiologic factors. Verdan of Lausanne (1944)



reverted to the toxic theory reporting a case of a man aged 40 years with a gastric ulcer following burns associated with spinal cord damage. He postulated that toxins might have acted on the cord and this in turn caused the ulcer by a nervous mechanism. The case of fatal perforated esophagus occurring four days after a severe burn in a woman, aged 29, treated with tannic acid and reported by Robson (1943) may be somewhat similar in its origin to Curling's ulcer. The perforation was 2 inches above the cardiac end of the stomach and the probability of inhalation of flames was not present. Rankin (1945) also reported an esophageal perforation following a burn.

Kellaway and his associates (1943) have published results of burn experiments using perfusion of a heated limb. Previously, Nagamitsu (1935) reported a vaso-depressor substance in the fluid perfusing a burned limb. In the first paper, Kellaway and Rawlinson, 1944, studied the effects of heat on the isolated lungs of guinea pigs ventilated with oxygen, air and nitrogen. In these experiments the degree of injury produced was estimated by the amount of histamine set free in the perfusate and drainage fluid expressed as a percentage of the total content of the perfused tissue. The histamine assay was done using strips of isolated guinea pigs' jejunum suspended in an oxygenated bath of Tyrode's solution at 35°C. Torantil neutralization was used as an additional means of proving that the substance was histamine. The experiments indicated that ventilation with pure oxygen neither increases nor decreases the histamine output to any extent, while heating to 45°C would produce an increase. These experiments essentially showed that in perfusion at least of the lung, anoxia does not cause gross injury and does not markedly aggravate heat injury in so far as histamine output is concerned. On the basis of these preliminary experiments the authors thought that they could give more credence to their subsequent ones mentioned below in which the factor of anoxia was not controlled. It can be said, therefore, that the liberation of histamine from the isolated lungs of the guinea pig is not significantly increased by anoxia more than that which attends the perfusion of an isolated organ with saline. The weakness in these experiments is that results obtained from the lung may not be applicable to those obtained from other organs.

In the second paper Rawlinson and Kellaway studied the liberation of enzymes from the perfused liver. The object of these studies was to ascertain the effects of cellular injury by moderate degrees of rise of temperature. Since they were especially interested in the liberation of enzymes, it seemed advisable before experimenting with complex structures such as limbs to study a relatively homogeneous tissue known to be rich in enzymes. They therefore perfused the isolated liver of the cat with saline at temperatures ranging from 38 to 50°C. Several substances were investigated. At 38°C none of them reached a maximum output until at least 19 hours. A rapid increase in output of alkaline phosphatase, esterase, proteolytic enzymes, and to a lesser degree of histamine as the temperature approached 40-46°C indicated that this is the critical level for heat injury. The maxima were reached in the following order: phosphatase, phosphate, proteolytic enzymes, esterase, and catalase. These time differences applied above 43° as well.

The Arrhenius equation in the form

$$\frac{d \ln k}{dT} = \frac{\mu}{RT^2}$$

involves the use of  $k$  as the rate constant of the process,  $T$  as the absolute temperature,  $R$  as the gas constant, and  $\mu$  as an empirical temperature characteristic. Rawlinson and Kellaway used this as modified to

$$\log \frac{a}{t} = \frac{\mu}{2.303R} \left( \frac{1}{T} + C \right)$$

where  $a$  and  $C$  are constants and  $t$  is the specified time. This equation should be satisfied if a straight line is obtained by plotting  $\log \frac{1}{t}$  against  $\frac{1}{T}$ . These authors assumed that for purposes of graphing at the temperatures studied, the degrees Centigrade could be substituted for the reciprocal of the absolute temperature. Thus, using  $1 + \log \frac{1}{t}$  as ordinates and  $^{\circ}\text{C}$  as abscissae, an ascending essentially straight line was found within the ranges of temperature studied ( $38\text{--}50^{\circ}\text{C}$ ) for the values of histamine and catalase. The alkaline phosphatase and esterase lines showed a marked rise from their previously straight course at about  $46^{\circ}\text{C}$ . This forms the basis for Rawlinson and Kellaway's assumption that plotting their values in this manner, there is a greater outpouring of these two substances from the perfused liver above  $46^{\circ}\text{C}$  than would be accounted for by increased cell damage due to perfusion at a higher temperature.

Thus, the rapid increase in the return of liberation of such substances as inorganic phosphate, alkaline phosphatase, esterase, proteolytic enzymes, and to a lesser degree of histamine, when the temperature approaches  $40$  to  $42^{\circ}\text{C}$  is considered to be greater than can be accounted for by the normal exponential increase of the processes operating at  $38^{\circ}\text{C}$ . On the other hand the liberation of catalase and histamine above  $38^{\circ}\text{C}$  is slower than that of the other constituents studied. The liberation process for catalase satisfies the Arrhenius equation and it therefore may be concluded that the rate-determining steps are characteristic of the enzyme. The gross deviation from the Arrhenius equation by alkaline phosphatase and esterase above  $44$  to  $45^{\circ}\text{C}$  demonstrates that inactivation is commencing. Catalase on the other hand shows no such destruction up to  $50^{\circ}\text{C}$ .

In the third paper, Kellaway and Rawlinson (1944) studied tissue injury by heat in isolated limb preparations. They believed that this offers a useful lead for the discovery of possible toxic products whether these be cell constituents or formed in the cells or tissue spaces by enzymatic action consequent upon heat injury. From the perfused hind limbs of the guinea pigs histamine was liberated from between  $45^{\circ}$  and  $50^{\circ}\text{C}$ . From the cat forelimb histamine was similarly liberated. In the early hours of perfusion a smooth muscular relaxing substance which inhibited the stimulating effect of histamine was present in the perfusate. No active adenylyl compounds could be detected between  $37.5^{\circ}$  and  $50^{\circ}\text{C}$ , but

cardiodepressant activity was found between 42° and 50°C. This was present also in the subcutaneous edema and the substance was heat stable and was not diminished by incubation with cat muscle or cat liver extracts. The hydrogenized concentration of the perfusate showed greatest increase at 44 to 45°C. The small output of inorganic phosphates at 37.5° to 41°C had superimposed upon it at the higher temperatures a much larger output. Alkaline phosphatase, lipase, and proteolytic enzymes were also set free above 41°C. Kellaway and Rawlinson concluded "The demonstration that lipase and proteolytic enzymes were set free from the perfused hind limb of the cat above 41°C gives color to the possibility that toxic products may be formed by enzyme activity in the tissue spaces."

17 *The liver lesion and tannic acid* Early reports noted a central necrosis of the liver in fatal burn cases, but did not implicate tannic acid as the causative factor. These reports included those of Wilson, Macgregor and Stewart (1938), Belt (1939), Zinck (1941), Buis and Hartman (1941), Duffin (1942), Boyce (1942) and others. Previous observers, including Bardeen (1898), Weiskotten (1919), Weimann (1927), Vogt (1929), and Hiroto (1934) (all cited by Harkins, 1942), had reported liver necrosis in burns, but such a damage was more diffuse and was not the typical marked central necrosis with polymorphonuclear neutrophile infiltration associated with the tannic acid cases and first reported by Wilson, Macgregor, and Stewart (1938).

The possibility that tannic acid might cause focal central necrosis of the liver was first postulated by Wilson in Great Britain in 1938. Wells of Hartford, Conn. reported the first experimental evidence that tannic acid is the cause of liver necrosis in burns before a panel discussion at the Clinical Congress of the American College of Surgeons in Chicago, October, 1940. In a memorandum submitted to the Burns Subcommittee (British) in May 1942, Wilson referred to the possibility that absorption of tannic acid might be a cause of toxemia in human cases of burns so treated. His experience in the Middle East, though admittedly inconclusive, had indicated that such toxemia was more common and severe in burned patients treated with tannic acid, and he described definite pathologic changes in the livers of patients who had died in the toxemic stage after tannic acid treatment. This action was shown to be true experimentally by Wells, Humphrey and Coll (1942) and later by Forbes and Evans (1943), by Baker and Handler (1943) and by Hartman and Romence (1943). Erb, Morgan and Farmer (1943) studied the postmortem findings in 61 cases of burns. Of these, 41 were tanned, with 25 (61 per cent) instances of liver necrosis, and 20 were not tanned, with 0 (0 per cent) instances of liver necrosis. Rosenthal (1942) working with mice, found that "significant increases of early mortality were produced by tannic acid solutions and ointment when applied to a scalded area." These observations seem to point rather conclusively to the potential danger of liver necrosis from tannic acid therapy. A closer study of the reported cases and experiments indicates that the tanning methods in which the active agent is kept in contact with the burned surface in a moist state for a long period of time (tannic acid jelly or baths) are especially dangerous. The increased

chances for absorption seem to be the influencing factor, as pointed out by Har-kins (1943) and Rae and Wilkinson (1944). It has long been known that acute toxemia may follow the wetting of a dry tannic acid eschar, and as Rae and Wil-kinson state, this too may be due to absorption of tannic acid after wetting rather than to liberation of other toxins. Rapid tanning as by tannic acid silver nitrate, where the agents dry within a matter of minutes or seconds, is less dangerous in this regard.

Rae and Wilkinson (1944) studied the liver function after burns in children by means of the levulose tolerance test. Treatment included silver nitrate, tannic acid jelly, and sulfonamide. Disturbance of liver function was greatest with tannic acid jelly and least with silver nitrate. These authors stated "There is now sufficient evidence, both clinical and experimental, to justify disuse of tannic acid as a local application for burns."

Wilson (1941) found that while injections of tannic acid were toxic to animals, silver nitrate in similar doses produced no such toxic effects. Cameron, Milton, and Allen (1943) produced the typical liver lesion by treating burned areas with tannic acid solutions. The degree of liver damage was related to the extent of injury and possibly also to its depth, since in goats, where the lesion corre-sponded roughly to a deep human burn, liver damage was less marked than in rabbits, where the injury was more superficial. Barnes and Rossiter (1943) obtained results somewhat similar to those of Cameron, et al. These Oxford authors found that when tannic acid and sodium tannate are injected intra-venously, they are fatal to mice and guinea pigs in doses of the order of 40 mgm per kgm. The fatal intramuscular dose was much greater. In rats sodium tannate given intramuscularly was more toxic than tannic acid. After applica-tion of tannic acid to the raw area produced by burning guinea pigs to the extent of a quarter of the body surface, there was slight but definite liver damage which was greater than that in the controls. Similar burns treated with tannic acid had a higher mortality than control burns of the same extent not so treated. In another paper, Clark and Rosseter (1943) studied liver function in rabbits after injection of tannic acid. After subcutaneous injection of 100 to 750 mgm of the drug per kgm., there was a depressor of liver function as measured by the galactose-tolerance test. This impairment of function was also observed after the intravenous injection of smaller doses (5-10 mgm /kgm) of tannic acid.

Cameron, Milton, and Allen (1943) concluded "There seems little doubt that even with not very extensive skin burns the application of tannic acid may be followed by its appearance and persistence in the blood and the development of slight or moderate liver damage. So far we have not had any fatal results [experimental animals: goats and rabbits], but this is most likely a question of extent of burn and amount of tannic acid applied. In none of our experiments has the blood level of tannic acid been high, the period of time over which tannic acid persists in the blood seems to be much more important in the production of injury." These authors agree with Robinson and Graessle (1943) that these effects are not due to gallic acid.

Robinson and Graessle (1943) recorded experiments on the toxicity of tannic

acid When applied to the deep subcutaneous tissue following surgical removal of the skin, tannic acid is lethal to mice, but not to rats or rabbits Liver damage shown by increased retention of bromsulphalein resulted in rabbits from subcutaneous tannic acid administration but not when it was applied to denuded surfaces Clark and Rossiter (1943) found that liver function, judged by galactose tolerance, is impaired more noticeably by subcutaneous than by intravenous injection of tannic acid, possibly because of long-continued absorption

Other papers concerning the liver and burns include those of workers in Boston, Oxford, and Los Angeles Muus and Hardenburgh (1944) (Boston) reported that the oxygen consumption of normal rat liver slices is increased when measured in lymph from the burned legs of calves or one dog as a medium The  $Q_O$  in lymph after burning was as much as 41 per cent higher than that in normal lymph A less noticeable increase was noted when serum collected after burning was used in place of lymph The material which caused the increase in oxygen consumption was found to be present in the ultrafiltrate from the lymph after burning In a later paper, Muus, Hardenbergh, and Drinker (1944) found that lymph from the burned legs of a series of dogs acted similarly, not only in increasing the oxygen consumption of rat liver slices but also of rat diaphragm muscle

Clark and Rossiter (1944) of Oxford studied the metabolism of rabbit liver slices after burning Anesthetized rabbits scalded over one-third of the body surface at  $70^{\circ}\text{C}$  for 30 seconds demonstrated a hemoconcentration at the end of 4 hours which had disappeared in 24 hours In these animals there was no change in the ability of the liver to oxidize alanine or sodium butyrate either 4 or 24 hours after burning, and there was no change in the  $Q_G^O$  and  $Q_O$ , measured in bicarbonate buffer After 4 hours there was a fall in the  $RQ$  (measured in phosphate buffer), rise in the  $Q_G^N$ , and fall in the ability of the liver to form glycogen from glucose, these functions all returned to normal within 24 hours After 24 hours there was a slight rise in the  $Q_O$ , measured in phosphate buffer, this not being observed after 4 hours These results are correlated with the time of onset and disappearance of hemoconcentration and it was concluded that they are probably secondary to the circulatory changes following the burn Wilhelm and Harkins (1944) also found that the  $Q_O$  does not change appreciably in rat-liver slices over a period of 15 hours following a scale of a greater degree than that which Clark and Rossiter's rabbits received (rat scald  $80^{\circ}\text{C}$ , 7 sec, over entire body surface except head)

The Los Angeles group working with Prinzmetal (Prinzmetal, Hechter, Margoles, and Feign, 1943 and 1944) reported that a principle obtained from liver is effective against shock due to burns, as tested on scalded etherized rats Isotonic saline solution, while helpful, was not as active in preventing shock as was the liver action The latter in turn was not identified with the antianemia principle This work of Prinzmetal and associates is to be correlated with that of Forbes and McConnell (1937) on a liver fraction protecting against liver necrosis from carbon tetrachloride or chloroform administration

Abandonment of tannic acid has not entirely eliminated burn toxemia, and the

question still remains whether selected burn cases may not under certain conditions be treated with advantage by this type of therapy. A more complete discussion of this important subject is made by Lee and Rhoads (1944). These authors point out the disadvantages and advantages of the method and leave its future place open for subsequent opinion to decide.

McClure, Lam, and Romence (1944) wrote an article entitled "Tannic acid and the treatment of burns. An obsequy." Coming from the same institution where the modern tannic acid treatment of burns had its birth in 1925, this report carried additional weight in indicting this mode of therapy. These authors stated "Liver necrosis had been reported in a considerable number of burned patients treated with tannic acid. Nonfatal cases frequently showed marked disturbance of the liver function in the acute phase of the burn. The liver lesion is easily reproduced experimentally. Wound healing experiments on animals and on human donor sites indicate that tannic acid retards healing considerably." In conclusion, the words of Cameron, Milton, and Allen (1943) are a succinct expression of now popular opinion: "It seems that all may not be well with the tannic acid treatment of burns."

**18 Sodium therapy.** Plasma (or albumin) and whole blood is the accepted treatment of burn shock. Recently, however, various investigators have reported efficacious results with sodium salts, especially by mouth. Hoitink (1938) found normal saline solution to be more efficacious in hemorrhagic shock as a blood substitute than glucose or gum acacia and concluded that its intravenous application is "preferable to the use of other artificial blood substitutes and to blood transfusion."

Rosenthal (1943), experimenting on mice, produced a standard burn by immersing the animals up to the neck in water at 70°C for a measured period of seconds. A large series of mice was thus burned and treated groups were compared with untreated controls as to mortality. Sodium chloride by mouth or intraperitoneally caused a significant reduction in mortality. Intravenous administration was less effective. Isotonic solution of sodium chloride by mouth was superior to hypertonic solutions. Sodium acetate, succinate, bicarbonate and lactate were as effective as sodium chloride. Mouse serum injected intravenously was slightly less active than equivalent volumes of 0.9 per cent sodium chloride orally. These results are of great interest especially as they do not agree with the present concept of the superiority of intravenous plasma or serum in the therapy of burn shock. The possibility arises that the results obtained on mice are not entirely applicable to man.

Fox (1944) used oral sodium lactate in the treatment of 17 burn cases. Chilled one-sixth molar sodium lactate was given at frequent intervals so that approximately 7 to 10 liters (10-15 per cent of body weight) was given the first 24 hours. Any vomiting was treated by administration of more fluid. Fox reported good results in this series of cases and since the time of this report has used the method in a much larger series of cases (personal communication, Sept. 1944). Cope (1944) discussed the rationale of Fox's method.

Prinzmetal, Hechter, Margoles, and Feigen (1943) in working on mice and rats also reported good results with sodium therapy. They stated "Nineteenth per cent solution of sodium chloride, when administered [intraperitoneally] in amounts equivalent to 5 or 10 per cent of the body weight, is definitely effective against burn shock when given either after or thirty minutes prior to trauma." Parkins, Koop, Riegel, Vars and Lockwood (1943) studied burn shock in dogs and stated "it appeared that saline alone [intravenously] was beneficial to these animals." Levine, Huddleston, Persky, and Soskin (1944) found in the so-called irreversible stage of hemorrhagic shock, whole blood supplemented with sodium bicarbonate and glucose (38 per cent mortality) was much better than whole blood alone (75 per cent mortality). This work implied that acidosis as well as a sodium deficiency may play a rôle. Coonse, Foisie, Robertson, and Aufranc (1935) also found alkali therapy to be of benefit in human traumatic and hemorrhagic shock and in experimental shock in dogs. On the other hand, Rosenthal (1943) found sodium chloride to be just as efficient in burn shock as is sodium lactate.

Moyer, Coller, Iob, Vaughn and Marty (1944) reported experiments on burn shock in dogs which tend to favor the view that acidosis may be a noxious factor in this syndrome. Their conclusions were: Saline-bicarbonate solution is more effective than Ringer's lactate solution, and Ringer's lactate solution is more effective than "isotonic" sodium chloride solution in prolonging life of anesthetized dogs when 10 per cent of body weight of these solutions is given intravenously before the animals are scalded (two-thirds body surface at 85°C for 30 sec). The presence of the bicarbonate ion or the potential bicarbonate ion in the lactate solution appears to be responsible for the superiority of the Ringer's lactate and the saline-bicarbonate solutions. The combination of massive transfusions of defibrinated blood and saline-bicarbonate solution by stomach was the only form of therapy employed that prevented shock without inducing complications that were incompatible with life. The other forms of therapy employed, namely (1) Saline-bicarbonate, 1 v, (2) saline-bicarbonate 1 v and serum 1 v, (3) serum 1 v and saline-bicarbonate by stomach, and (4) defibrinated blood 1 v and water *ad libitum*, prolonged life and in a number of instances prevented shock. But all the animals that did not die of shock during the first 24 hours died later of complications that seemed to be related to the therapy rather than to the trauma.

The work of Wilson, Macgregor and Stewart (1938) is of interest with regard to sodium therapy. These authors reported that a considerable fall in the level of serum sodium occurred in certain of their patients following extensive burns or scalds. Lowdon, McKail, Rae, Stewart and Wilson (1939) repeated this observation and also found that the serum sodium is rapidly restored to a normal level by desoxycorticosterone acetate. They then investigated these changes by animal experiments. Scalds were produced in cats under nembutal anesthesia by immersing the hind limbs and posterior third of the trunk in water at about 90°C for 5 seconds. Subsequently the characteristic condition of "shock" supervened and the animals died in from 45 minutes to 4 hours after injury. After scalding, the level of serum sodium of arterial blood and of sodium in

cerebro-spinal fluid steadily declined, while the sodium in the red blood corpuscles tended to increase. These changes were not prevented by previous section of the spinal cord, decerebration, removal of the kidneys or of the suprarenal glands.

In control experiments without scalding the procedures of repeated withdrawal of blood samples, suprarenalectomy, nephrectomy, spinal cord section and decerebration produced no more than a small and transient fall of serum sodium. It seemed to these authors therefore, that the loss of sodium from serum and cerebro-spinal fluid was independent of any action of the kidneys or central nervous system and that it was not due to impairment of functional activity of the suprarenal cortex since the serum sodium did not fall after removal of the suprarenal glands in experiments of similar duration. It seemed logical to them to conclude that the diminished content of serum sodium was not merely a consequence of hemolysis of red cells which was an invariable sequel of scalding.

The following facts suggested to Lowdon and his associates that the sodium was being lost into the scalded tissues.

(1) No significant fall in serum sodium occurred if the circulation to the scalded area were effectively occluded before scalding.

(2) The serum of venous blood from scalded skin contained less sodium than did the serum of arterial blood.

(3) When the isolated hindquarters of the cat were perfused with heparinized blood a sharp decline of plasma sodium began about 1 hour after scalding and the plasma of outflow blood had a significantly lower sodium content than the plasma of inflow blood.

They tested this theory by estimations of tissue sodium but the evidence was incomplete.

Lowdon, et al, next tested the action of desoxycorticosterone acetate in restoring the level of serum sodium after scalds in animal experiments, 45 minutes after intramuscular injection of desoxycorticosterone acetate the serum sodium had returned from a low to a normal level. How and from what source the sodium is mobilized was not determined.

Other changes in the blood were noted after scalding. Hemolysis of red cells has been mentioned, it precluded satisfactory estimations of serum potassium. The chlorides of serum (or plasma), like the sodium, diminished, but the chlorides of whole blood increased. A curious feature was that when, prior to scalding, the cat was decerebrated or the spinal cord sectioned, then the serum chlorides, but not the serum sodium, rose instead of falling. Urea and non protein nitrogen increased both in serum and whole blood, and there was usually a moderate rise in corpuscular concentration. Finally, these authors found that substantially the same changes in blood chemistry which followed scalding were noted when shock was produced by hammering the thigh muscles of the cat.

The use of sodium therapy in burns is one of the chief points of interest at the present time. It would seem unwise, however, to discard the proven advantages of plasma treatment, and better to use the administration of sodium salts as an adjunct to plasma therapy rather than as a substitute for it.



19 *Burn anemia* Most serious burns show a biphasic hemoglobin concentration curve. Early hemoconcentration is followed by a progressive anemia which begins to develop on about the fifth day and remains troublesome as long as granulating surfaces remain unhealed. Actually the anemia may have its onset at the time of the burn and the subject of "early masked anemia" was reviewed *in extenso* by Harkins (1942). Early in the course of the burn the erythron may be reduced, but such a diminished volume is concealed by the even greater decrease in the plasma volume. Actual red blood cell destruction with resultant hemoglobinemia and hemoglobinuria may occur at the time of the burn.

In an article describing a thrombocytopenia which occurred in 12 out of 13 burn cases in from 7 to 57 hours after the burn, MacDonald, Levenson, Davidson, Tagnon, and Taylor (1944) stated that "hemoglobinemia was present for varying periods of time in all of the 13 patients." Cope and Rhinelander (1943) reported 9 cases of hemoglobinuria in patients burned in the Cocoanut Grove disaster. Numerous older writers on the same subject including Lesser (1880), Pfeiffer (1905) and others are reviewed by Harkins (1942) and by Shen, Ham, and Fleming (1943). The latter article is one of the best presentations of this particular subject. These authors studied 14 cases of burns and found hemoglobinuria in 10 of them. Hemoglobinemia during the first 20-36 hours was present in 8 cases with values ranging from 65 to 215 mgm of hemoglobin per 100 cc of plasma. In 6 patients who had shown hemoglobinuria, pathologic examination of the kidneys showed hemoglobin casts. The osmotic fragility of the red cells was significantly increased above normal in 7 patients. In 4 patients tested the osmotic fragility of the red cells was increased in 3 when determined within a period of a half to 3 hours after the burn. Spherocytes were significantly increased in several cases and were 3 per cent or above in 4 instances. *In vitro* studies on heated blood showed no alterations with temperatures as high as 46°C. Above that point changes occurred.

In patients with severe or moderately severe thermal burns, the red cells examined promptly after the burns exhibited changes in morphology and osmotic fragility similar to those obtained by the injection into dogs of the animals' own erythrocytes heated *in vitro* to approximately 53°C. The increased osmotic fragility of heated red cells apparently results from conversion of the normal biconcave erythrocytes to more nearly spherical forms by a process of progressive fragmentation. The mechanism by which heat causes fragmentation of red cells with increased osmotic fragility *in vitro* occurred through a mechanism of swelling and osmotic hemolysis in the isotonic plasma of the animal.

Shen, Ham, and Fleming concluded "In thermal burns a significant number of erythrocytes may be destroyed by heat, probably depending on the temperature attained by the blood, the duration of heating and the volume of blood subjected to these conditions." Netsky and Leiter (1943) noted in their experiments that "intravascular hemolysis and escape of hemoglobin into the lymphatics is a consequence of severe burn."

In general, it can be said that hemoglobinemia and associated hemoglobinuria

are often present during the first day or so after a very severe burn. Shen, Ham, and Fleming (1943) showed that such hemoglobinemia usually results from direct trauma to red cells in the capillaries of the burned skin, while the precipitation of hemoglobin in the kidneys may contribute to the oliguria that often is noted. Lund (1943) gave the following advice in this regard: Patients with severe burns "should be catheterized at once and hemoglobin sought in the urine. Also the first blood specimen taken for hematocrit determinations should be used to test for hemoglobinemia. If either hemoglobinemia or hemoglobinuria are present, the patient should be alkalinized with intravenous injections of 40 cc of 1 M lactate to each 500 cc of plasma or an equivalent amount of sodium bicarbonate. As soon as an alkaline urine is established, the sodium lactate administration should be stopped." Rosenthal (1943) and Fox (1944) also found alkaline solutions to be of benefit in burns as discussed under sodium therapy. Hemoglobinuria was also noted by Lucido (1940) and many others. Bingold (1944) of Nurnberg believes that a definite renal causative factor may exist in these cases. Co Tui, Wright, Mulholland, Barcham, and Breed (1944) pointed out that the protein in the hemoglobin may account for some of the early urinary nitrogen losses reported in the literature. Olson, Walker, and Necheles (1944) also noted a hemoglobinemia in dogs burned under nembutal anesthesia. In one of their animals, the plasma contained 1385 mgm per cent of hemoglobin 4 hours after the burn and in another instance, 45 minutes after the burn the plasma contained 3680 mgm per cent of hemoglobin. They concluded "The severity of the anuria in all burn experiments frequently could be associated with the degree of hemoglobinemia."

The work of Mendelsohn and Rossiter (1944) on the subcutaneous temperatures in moderate temperature burns may have a bearing on the production of hemoglobinemia from overheating of blood. Temperatures were measured with a copper-constantin thermocouple inserted through a hypodermic needle under a burned area of guinea pigs. One minute application of a burning iron at 70°C caused subcutaneous temperatures to rise to an average of 54°C, sufficient to injure red blood cells. Harkins and Harmon (1937) also made observations on the insulating power of living skin.

Recent observations by Moyer, Collier, Iob, Vaughan, and Marty (1944) studied the early masked anemia in burns from the therapeutic standpoint. Anesthetized dogs were immersed so that two-thirds of the body surface was scalded at 85°C for 30 seconds. They concluded that hemoconcentration per se is not harmful and "the hemoconcentration of severe thermal injury does not contraindicate the transfusion of whole blood." They found whole blood to be superior to serum and "the combination of massive transfusions of defibrinated blood and saline-bicarbonate solution by stomach was the only form of therapy employed that prevented shock without inducing complications that were incompatible with life."

Ham (1944) reported "The dilatation and congestion of both the smaller and larger blood vessels associated with the various types of burns studied in these experiments, together with the small hemorrhages that were not infre-

quently observed, serves to emphasize that extensive burns would tend to abstract significant quantities of whole blood from active circulation in addition to the plasma that is lost by leaking away from the smaller vessels "

Secondary anemia is a frequent occurrence after severe burns, in many cases coming on after the fifth day. It may be due to three chief causes: a) reabsorption of fluid, b) primary destruction or increased fragility of red cells due to the burn (Shen, Ham and Fleming, 1943), and c) continued destruction and slow formation of red cells due to sepsis, bleeding from granulating surfaces, and malnutrition in the late stages. The treatment consists in blood transfusions, a high protein, high vitamin diet, iron, and, in some cases, liver.

**20 Burn hypoproteinemia** Early hypoproteinemia is rare although the total amount of plasma protein in the vascular tree may be drastically reduced. Such early hypoproteinemia when it does occur is often associated with excessive intravenous treatment with non-colloid solutions. Late in the course of burns hypoproteinemia is common and when granulating surfaces are present it tends to complete a vicious circle. The hypoproteinemia persists as long as the granulating surfaces remain and the latter will not heal as long as the hypoproteinemia interferes with their nutrition.

Hirshfeld, Williams, Abbott, Heller, and Pilling (1944) pointed out that patients who are moderately or severely burned may excrete more nitrogen in the urine than can be administered orally as protein without forced feeding. Second degree burns may show a temporary nitrogen deficit, but since most patients can stand such deficits for a short time it is in the third degree burns that maintenance of a high protein intake is essential. After three weeks of excessive protein catabolism such patients are still not only excreting abnormally large quantities of nitrogen in the urine, but are also losing considerable protein in the exudate from the burned surface. Using a specially prepared dressing, these authors studied the nitrogen loss from the burned surface. Closely woven glass cloth covered by a thick layer of cellucotton, and this in turn by an impervious covering of vinylite plastic bandage, was used to collect the exudate. Complete nitrogen balance studies were done on 6 patients with burns and it was found that from 3 to 35 per cent of the total nitrogen output occurred from the burned surface. These authors concluded "Nitrogen continues to escape in significant quantities in the burn exudate until epithelization has occurred. This is an additional reason for the early skin grafting of large third degree burns."

Co Tui (1945) determined the extent of protein loss in burn exudates. In most cases nitrogen-free slabs of cellulose sponge were used for collection of the exudate. Ten 24 hour collections were made in 7 burn patients, at times varying from the second to the 94th day after the burn. The protein loss varied from 9.4 to 56.7 grams per day. This is equivalent to 150 to 945 cc of plasma per day and is thus seen to be a tremendous loss. In four instances the area of the burn was determined by polar planimetry and the protein loss expressed as milligrams lost per square centimeter of area. Such loss varied from 5.6 to 20.4 mgm of protein per square centimeter of open burned surface. Co Tui concluded "The nitrogen losses were, in some cases, of sufficient magnitude to jeopardize recovery."

Urinary loss of excessive amounts of protein has been noted by many authors. Lucido (1940) reported that his patient had a high urinary excretion of nitrogen for the first 25 days following the burn. Browne (1942) studied the nitrogen output in three cases of burns. On a regular diet, all showed an elevated urinary output to as high as 28 grams per day, gradually decreasing to normal on the 40th to the 52nd day. Taylor, Levenson, Davidson, Adams, and McDonald (1943) reported an excretion of as much as 45 grams of nitrogen (equivalent to 250 grams of protein) a day. Cope, Nathanson, Rourke and Wilson (1943) reported lower values for the urinary excretion of nitrogen in burns and attributed the negative nitrogen balance in these patients to a low intake. Deaver, Cronkite, and Phillips (1944) also reported an abnormal nitrogen metabolism in a burned patient. Taylor, Levenson, Davidson, Browder, and Lund (1943) in a later paper, reported one case of a patient with 45 per cent of the body surface involved by a third degree burn who excreted as much as 34 grams of nitrogen in the urine with an average of 28 grams daily the first week. These authors found that correction of such a difference by high protein feeding failed to bring the patient into true nitrogen balance because of incalculable losses from the burned surface. However, heroic intravenous and tube feeding of about 300 grams of protein a day would restore his true balance, although at the time of writing he was still one-third below his normal weight.

21 *Nutritional factors* Co Tui, Wright, Mulholland, Barcham, and Breed (1944) discussed the problem of nutrition in burns. These authors pointed out the possibility that the early protein loss reported by many writers may be partly accounted for by hemoglobinuria. They found an apparent mathematical relationship between the extent of the surface burned and the amount of nitrogen required to maintain nutrition. They pointed out that skin grafting in these patients with protein deprivation is especially liable to lead to shock. Although not stated in their paper, it should be mentioned that such cases should be skin grafted directly on top of the granulations, because removal of the latter may precipitate shock. Co Tui and his associates also reported that with adequate protein nutrition the necessity for blood transfusions was reduced to a minimum. This is in agreement with observations of Evans (1944).

Elman, Charnas, and Davey (1943) used an enzymic hydrolysate of casein containing a mixture of amino acids and polypeptides in the treatment of protein-depleted dogs and found that 1.75 grams of nitrogen per kilogram per day produced a positive nitrogen balance. This aspect of burn treatment was discussed in a recent paper by Aguilar (1944).

Taylor (1944) and Taylor, Davidson, and Levenson (1944) discussed the problem of protein nutrition in serious thermal burns. In the latter paper they stated "It is a safe minimum margin that the severely burned patient will require as a minimum 2 to 3 grams of protein and at least 60 calories per kilogram of body weight from the earliest possible moment if weight loss and protein deficiency are to be prevented." However, when protein deficiency is already present, more urgent therapy is required, as 500 grams of protein by intubation and by vein may be needed per day to accomplish this result in a reasonable time. *It seems obvious, therefore, that the successful treatment of patients with a*

*potential nitrogen deficiency lies in an early appreciation of the nitrogen demand and the immediate institution of remedial measures to prevent its occurrence "*

Clowes, Lund, and Levenson (1943), reporting on 150 cases of burns, 109 of whom were victims of the Cocoanut Grove disaster, epitomized the need for nutritional care of burns as follows "All patients with 10 per cent of surface area or more involved in third-degree burns became serious nutritional problems because of the loss of nitrogen in the urine and from the surface, and because of the increased nutritional requirements resulting from infection with fever "

**22 Metabolic factors** Cope, Nathanson, Rourke, and Wilson (1943) pointed out that a disturbance in nitrogen balance is one of the most notable features after thermal burns. This subject has already been discussed in the last two sections. A decreased metabolic rate with resultant anoxic changes in the presence of severe shock is another important metabolic alteration. The reviews of McClure (1939) and of Lam (1941) are pertinent to this aspect of the subject.

Recent observations of Harkins, Long, and associates (1945) concerning metabolic changes are as follows. In all instances burns (scalds) were produced by immersing white male rats of standard size in hot water of constant temperature for a definite period of time. From such an injury there resulted a rise in plasma amino nitrogen, a slight rise in plasma ammonia nitrogen, a slight rise in liver amino nitrogen, practically no change in liver ammonia nitrogen or liver amide nitrogen, and no early decrease in oxygen uptake of liver slices but a late slight decrease, as measured by the Warburg apparatus. The significance of these findings is best explained by correlating them with the observations of Engel, Winton, and Long (1943) on hemorrhagic shock. It is enough to say, however, that many of these changes appear to be of metabolic origin rather than directly produced by the burned area.

Cope, Nathanson, Rourke, and Wilson (1943) found in their burn cases that the 17-ketosteroid excretion was elevated during the period corresponding to the negative nitrogen balance. This observation brings the subject of metabolic disturbances in burns to a consideration of a possible relationship to endocrine factors, as discussed in the next section. The Boston group reported an abnormal growth of hair in six women patients hospitalized for more than three weeks, despite low 17-ketosteroid levels.

**23 Endocrine factors** Early observations indicating that adrenal damage is common in burns are reviewed by Harkins (1942). Cope and his associates (1943) noted the increased 17-ketosteroid excretion as well as an abnormal growth of hair in the six women hospitalized for more than six weeks, despite a low 17-ketosteroid level, referred to above. These and other observations have suggested a possible endocrine disturbance in burns. Recent work of Harkins, Long and associates (1945) has shown the presence in burns of not only a disturbance of one gland but an interrelationship between two glands. After the production of standardized scalds in anesthetized rats, there was a significant fall in adrenal total cholesterol (e.g., from a normal of 4.2 mgm. per 100 mgm. of adrenal weight to as low as 0.7 mgm.). This fall in adrenal total cholesterol did not occur in hypophysectomized rats, indicating that the effect of the burn may not be di-

rectly on the adrenal gland but is mediated by way of the pituitary possibly by a stimulation of production or outpouring of adrenotropic hormone

After burning there is a rise in blood sugar, both in man (Underhill, et al, 1923, Davidson, 1925, McIver, 1933, and Lambret, et al, 1936) and in experimental animals (Schreiner, 1920, Greenwald and Eliasberg, 1926, Lundberg, 1929, Beard and Blalock, 1931, Slocum and Lightbody, 1931, and Stolfi, 1936) Taylor, Levenson, and Adams (1944) reported observations on the abnormal carbohydrate metabolism in human thermal burns, emphasizing especially the occurrence of hyperglycemia. In a group of 35 consecutive burn patients none of whom were known diabetics, admitted to the Boston City Hospital, 20 were in a hyperglycemic state on entry. "The initial Folm blood sugar level varied from 132 to 352 mgm per 100 cc. The occurrence of hyperglycemia was directly related to the extent of the burn. The hyperglycemia persisted over a period of hours in all cases, and over a period of days in some. None of the patients studied showed a return of an elevated blood sugar level to normal within 12 hours." Associated with the hyperglycemia was an initial decrease in carbon dioxide combining power and a lacticacidemia in certain cases. Thirteen of the 21 cases of hyperglycemia had associated hemoconcentration. While such blood sugar responses may be due to the pancreas, they may also be caused by hepatic involvement.

Clark and Rossiter (1944) working on rabbits and rats, also studied carbohydrate metabolism after scalds. Their observation can be summarized as follows. Burning caused a rise in the blood sugar of both rats and rabbits anesthetized with ether. In rabbits, this rise was greater in well fed animals than in those starved for 24 hours and had completely disappeared 24 hours after burning. In both species, adrenaline injections produced a similar hyperglycemia. In rats, whose adrenals had been removed, there was no rise in blood sugar after burning. Rats had a higher blood lactic acid one hour, but not 3 hours, after burning, adrenaline injections produced similar changes in blood lactic acid. Both 1 and 3 hours after burning, there was a fall in the glycogen content of the whole carcass (chiefly muscle glycogen) of rats. Similar changes were observed after adrenaline injections. The carcass glycogen of burned adrenalectomized animals remained normal. Rabbits, deprived of food for 24 hours and burned, had a lower liver glycogen, both 4 and 24 hours after burning, than the controls, which had been merely anesthetized. On the other hand, burning produced no change in the liver glycogen of either normal or adrenalectomized rats starved for 24 hours, but the injection of adrenaline caused a slight rise. Liver slices from rabbits killed 4 hours after burning formed glycogen from glucose less readily than slices from anesthetized control animals. Slices from similar animals, killed 2 to 4 hours after the injection of adrenaline which produced a similar degree of hyperglycemia, formed glycogen normally. Liver brei from burned rabbits did not break down glycogen either by phosphorylase or amylase activity, any more rapidly than a similar brei from control animals. There was no increase in the amylase activity of serum of rabbits either 4 or 24 hours after burning. There was a fall in the ascorbic acid content of the adrenal gland of

rabbits, both 4 and 24 hours after burning, but no change in the ascorbic acid of the liver Harkins (unpublished data) has found a similar fall in adrenal ascorbic acid after burning in rats

Clark and Rossiter also found that injection of 5 ml of isotonic ammonium chloride intraperitoneally into rats caused a rise in blood sugar of the same order as that observed after burning, but the plasma alkali reserve, after such an injection, was much less than that observed after burning Intraperitoneal injection of 5 ml of isotonic sodium bicarbonate did not influence the burn hyperglycemia any more than a similar injection of 5 ml isotonic sodium chloride They concluded that acidosis is thus not a major factor in the production of rat burn hyperglycemia Well fed rats were no better able to survive burning than similar animals starved for 24 hours, leading to the assumption that a high blood sugar plays no striking rôle in the protection of rats from the ill-effects of burning However, as a practical measure, it was suggested that glucose might be of benefit in the treatment of burns because of glycogen depletion that may result in such cases The source of the blood glucose seemed undoubtedly to be chiefly muscle glycogen, and it was postulated that it is mobilized by a mechanism similar to the Cori cycle muscle glycogen is mobilized in the form of lactic acid, which, in the liver, is transformed, possibly by way of glycogen, to glucose Finally, these authors concluded that, as is true for other types of hyperglycemia, there are two mechanisms contributing to the high blood sugar which follows burning

(1) The liberation of adrenalin from the adrenal glands

(2) Some other process or processes, either stimulating hepatic glycogenolysis or inhibiting glycogenesis

*Some Unsolved Problems* Much recent progress has been made in elucidation of the problem of thermal burns due to the stimulus of the present war Numerous important and practical questions based on the 23 items discussed in this paper still remain unanswered A few of these are listed as a stimulus for future research

- 1 Is a primary debridement of an early burn necrotic lesion advisable?
- 2 How can burn infection be controlled?
- 3 Should fresh burns always be cleansed?
- 4 Can the separation of burn sloughs be hastened or should that process be allowed to run its natural course?
- 5 The same question can be asked concerning the healing of burn wounds
- 6 What are the relative advantages of plasma and of whole blood in the treatment of burn shock?
- 7 Is sodium therapy of specific value in the treatment of burn shock and/or of burn toxemia?
- 8 Does burn toxemia exist, and if it does, how can it be controlled?
- 9 How can burn anuria be controlled and is it toxic in origin?
- 10 What is the relation between the continuous stupor or delirium of some serious burns and the local lesion?
- 11 Is the treatment of burn anemia and hypoproteinemia a simple matter of adequate protein feeding or do the two conditions represent separate problems, each of a more complex nature?

12 Exactly what are the metabolic and endocrine changes in burns and do they offer any previously unconsidered leads to treatment?

Expressed succinctly, burn shock, burn toxemia, and burn sepsis still represent the three most important obstacles to the recovery of a burned patient

#### SUMMARY

Some of the more important local alterations and processes in burns considered in this article include necrosis, edema, increased lymphatic flow, separation of burn sloughs, and healing and regeneration. General problems discussed include the use of morphine and anesthesia, burn shock, toxemia, anemia and hypoproteinemia, plasma and sodium therapy, the influence of environmental temperature on recovery and certain metabolic and endocrine relationships in burns.

Burns form a prime example of a type of trauma with marked local and wide spread general effects. In considering either the mechanism of any alteration associated with burns or the treatment of any phase of burns, both the local traumatic lesion and the general disturbances of the burned patient must be considered. Burn therapy should be a continuous process with correlation of the local, general, and skin grafting phases.

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# PHYSIOLOGICAL REVIEWS

VOL 25

OCTOBER, 1945

No 4

## CONTROL OF THE SECRETION OF ANTIDIURETIC HORMONE FROM THE PARS NERVOSA OF THE PITUITARY GLAND

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Since the publication of the observations of Frank (52), v den Velden (186) v Korschegg and Schuster (103) and Motzfeldt (139) it has many times been confirmed that extracts of the posterior lobe of the pituitary gland contain an antidiuretic rather than a diuretic substance as had originally been thought (116). This review is written from the standpoint that the antidiuretic substance is a hormone (ADH) formed in the pars nervosa of the pituitary gland, that it is liberated into the circulation, and controls the rate of loss of water by a specific action on the kidneys. That this is so is proved by many facts, of which the chief are briefly as follows. A potent antidiuretic substance can be extracted from all normal mammalian posterior lobes (59), glands in which the pars nervosa is atrophied, but the pars intermedia is normal, are practically inactive (50) (51), diabetes insipidus can occur in the presence of a normal pars intermedia (51), the antidiuretic substance is effective in the minute quantities typical of hormones (75) (146) (180), the antidiuretic action persists after denervation or isolation of the kidneys and is effective in the great majority of cases of diabetes insipidus. This is not the place for further discussion on this aspect of the subject. References to the literature may be found in Van Dyke (184), Fisher, Ingram and Ranson (51) and in volumes XVII and XX of the Research Publications of the Association for Research in Nervous and Mental Disease (151) (89). This review is concerned with those observations and experiments which help us to understand how the liberation of the ADH from the gland is regulated.

When referring to that part of the pituitary gland developed from the nervous system the term pars nervosa will be used. The term posterior lobe will be used where the pars nervosa together with the pars intermedia is meant.

As yet we have but scant knowledge of the means by which the chain of events is initiated which leads to an alteration in the rate of excretion of water by the kidneys. Possibly the factor involved is a change in concentration of water in the blood and tissues in relation to one or more dissolved substances. In support of this possibility are the findings of Priestley (153), (154), Margaria (121), Smirk (171), Heller and Smirk (83) and Baldes and Smirk (3) that after the ingestion of water measurable changes occur in the electrical conductivity and water concentration of the blood. That the composition of the blood rather than of the tissues is important as a stimulus seems probable from the work of

Baird and Haldane (2) who found that water diuresis could be induced at a time when the tissues contained an excess of salt Baldes and Smirk (3) observed what was apparently an adaptation to changes in the water concentration of the blood They found that if the osmotic pressure of the blood was raised slowly, ingested water resulted in a diuresis although the total osmotic pressure was not below normal limits, and conversely that a slow decrease in the osmotic pressure of the blood did not necessarily lead to a diuresis There seemed to be a relation between the rate of application of the stimulus and the response

By what structures in the pars nervosa the ADH is produced is uncertain, whether from the specialised neuroglia cells, the pituicytes (18), or at the nerve terminations (155) That the ADH is made in the pars nervosa and not in the neighbouring pars intermedia is probable since no antidiuretic substance can be extracted from a posterior lobe in which the pars nervosa is atrophied when the pars intermedia is normal (50) (73) (74)

The next question to be answered is whether the pars nervosa is regulated by some local change in its environment, such as the composition of the blood flowing through it, or indirectly by the nervous system and a distant receptor organ The site of the receptors which respond to changes in the water content of the blood is still undetermined, but many workers since C and M Oehme (144) have shown that neither the renal nor the splanchnic nerves subserve this function (101) (163) (194) Janssen (94) showed that the spinal nerves below the lower cervical region were not involved, and both he and Motzfeldt (139) excluded any participation of the vagus below the mid-cervical region The supraoptic and paraventricular nuclei, which, as will be described later, supply most of the nerve fibres going to the pars nervosa, have an unusually rich capillary supply in particularly close contact with the nerve cells It has tentatively been suggested that these nerve cells may be stimulated by alterations in the composition of the blood and that they may respond by bringing about changes in pars nervosa activity (49) This is only a suggestion and is not supported by any experimental evidence Vasquez-Lopez (185), on the other hand, believes the gland itself to be a sensory organ and bases this opinion on the arrangement and large number of nerve fibres in relation to the number of cells, the appearance of the cells and the vascular pattern He suggests no reason for the presence in the posterior lobe of a potent antidiuretic substance If indeed the pars nervosa be merely a sensory organ then the interpretation of many well-proved facts becomes exceedingly difficult If, on the other hand, the pars nervosa be an endocrine gland under the control of the nervous system then many anatomic, experimental and clinical observations may easily be correlated Anatomically, Cajal's (23) observation that the pars nervosa has an ample nerve supply arising from hypothalamic cells (67) (68) has frequently been confirmed Experimentally, injury to certain parts of the central nervous system brings about a condition in every way similar to diabetes insipidus more effectively than does injury localised to the pars nervosa itself (24), Marx (123) demonstrated that water diuresis may occur as the result of suggestion during hypnosis, exercise brings about an inhibition of diuresis due to its emotional element (164), certain

sensory stimuli can inhibit water diuresis (179), the rate of excretion of water is markedly affected by the exhibition of a number of narcotics (53). Clinically, Leschke's review (107) presents a good case for the involvement of the central nervous system in cases of diabetes insipidus.

**ANATOMY** First the anatomical findings on the innervation of the posterior lobe will be considered. The general conclusions to be drawn are as follows. The pars nervosa is richly innervated by fibres from the supra optic, paraventricular and certain other hypothalamic nuclei. The supra optic nuclei and

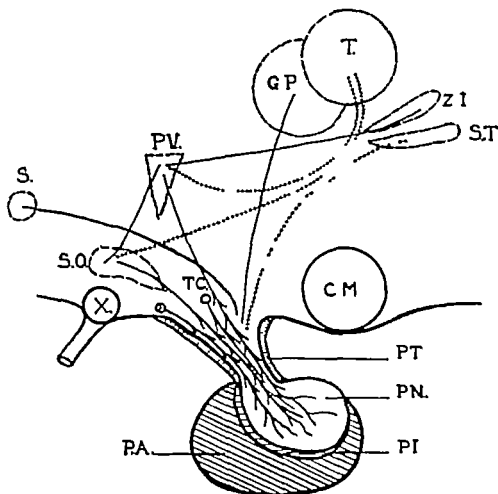


Fig 1 Diagrammatic representation of some of the nerve paths afferent to the hypothalamus and pars nervosa.

*CM* mammillary body *GP*, globus pallidus *PA* pars anterior *PI*, pars intermedia; *PN* pars nervosa, *PT* pars tuberalis *PV* paraventricular nucleus *S*, nucleus in septum pellucidum *SO* supra-optic nucleus *ST* subthalamus *T*, thalamus *TC* tuber cinereum *X* optic chiasma *ZI* zona incerta.

Dotted lines, periventricular fibres

tracts together with the pars nervosa appear to be a single functional unit since injury to any one part of the system results in a possibly proportional atrophy of the other parts, the same may be true of the paraventricular nuclei and their pituitary connections. The hypothalamic nuclei are, in their turn, linked with subthalamus and thalamic nuclei and through these with the sensory and motor systems and all parts of the cerebral cortex. The posterior lobe is also supplied with sympathetic and possibly parasympathetic fibres. Thus, there are many possible routes for the nervous control of posterior lobe function. The efferent



pathways from this area need not be considered here. Some of the work on which this general statement is based is described below. For a fuller account of the literature the reader is referred to (51) (89) (151) (184).

Krause (104) was the first to describe nerve fibres and endings in the *pars nervosa*. Croll (32) remarked on the abundance of the nerve fibres in the *pars nervosa* and suggested that as their number appeared to be excessive in proportion to the blood vessels present, their function was secretory rather than vasomotor. Bucy (18) reported the presence of end-bulbs at the terminations of the nerves. This was confirmed in cats by Hair (70). Rasmussen (155) has shown that in the human *pars nervosa* there are at least 50,000 non-myelinated nerve fibres.

Cajal (23) first described in the pituitary stalk of the rat a thick non-medullated plexus of nerve fibres terminating in the *pars nervosa*. Greving (67) (68) showed that in man this same tract originated in the supra-optic nuclei and passed to the *pars nervosa*, and further, that fibres from the paraventricular nuclei travelled to both the supra-optic nuclei and tuber cinereum. Pines (150) likewise drew attention to these tracts in man. Stengel (178) observed in cats and dogs, these and certain other hypophyseal fibres whose origin was probably the caudal part of the supra-optic nuclei and cells in the antero-lateral part of the tuber cinereum. Kary (98) and Lewy (110) reported retrograde degeneration in the supra-optic nuclei of dog and man following injury to the *pars nervosa*. Broers (15) confirmed this and showed further that in dogs lesions of the supraoptic nuclei resulted in atrophy of the *pars nervosa*. This has been confirmed for man, dogs, cats and monkeys (71) (81) (118) (148) (157) (158) (161). In particular must be mentioned the careful and detailed work of Fisher, Ingram and Ranson, fully described in their monograph (51). Mogilnitsky and Podljaschuk (133) attacked this problem by a different method. They subjected the posterior lobe to irradiation in doses far below those to which nerve cells are sensitive and found that the supra-optic tracts and nuclei suffered a retrograde degeneration which appeared to be proportional to the damage to the *pars nervosa*. A comparable supra-optico-hypophyseal tract has been found to be present in many groups of vertebrates (33) (96).

The paraventricular nuclei also are closely linked with the *pars nervosa*. Greving's (67) observation of fibres from these cells to the supra-optic nuclei and the *pars nervosa* has already been mentioned. Pines (150) confirmed this finding. Clark (30) reported the existence of similar nerve paths in man. Biggart and Alexander (9) produced lesions with the coagulating diathermy in the anterior hypothalamus of dogs and observed that in those developing diabetes insipidus there was some loss of paraventricular cells. This was confirmed in rats by Rasmussen (156) but not in dogs when the lesion was limited to the stalk or processus infundibuli. Others have found that in dogs, provided the *pars nervosa* removal be nearly complete, there is a loss of paraventricular cells (81) (148). Rasmussen and Gardner (157) observed some loss of paraventricular cells in man five months after stalk section.

There are afferent paths to the hypothalamus from the thalamus, particularly from the dorsal thalamic nuclei, and the subthalamic nuclei. Woollard (196),

following lesions in the subthalamus of a cat could trace degenerated fibres into the hypothalamus. The periventricular fibres appear to form a two-way link between the thalamus and the hypothalamus (31) (80) (162). Certain of these periventricular fibres terminate in the supra-optic and paraventricular nuclei (31) (116). Greving (66) described a tractus pallido-hypothalamicus but was uncertain of its exact termination. Degenerative changes have been reported in the globus pallidus of a man who had had diabetes insipidus (8). Loo (113) observed a tract from the zona incerta, some of whose fibres ended in the paraventricular nuclei, and also noted a well marked septo-hypothalamic tract whose exact termination could not be seen. Through these thalamic and subthalamic centres all regions of the cerebral cortex are linked with the hypothalamus (55) (56) (129) (130) (131) (132) (190).

There is also a sympathetic supply to the pars nervosa which originates from the plexus surrounding the internal carotid artery (36). Brooks and Gersch (16) removed the superior cervical ganglion in young rats and could find no change in the fibre pattern of the pars nervosa following this procedure. Hair (70), on the other hand, found some loss of fibres following cervical sympathectomy in cats and put forward the suggestion that the surviving fibres were parasympathetic in origin.

**EXPERIMENTAL AND CLINICAL OBSERVATIONS** This mass of anatomical evidence is meaningless unless experiment and observation show that these pathways are used, and in what direction. There are a number of conditions which may bring about a change in rate of renal water excretion due either sensibly or presumably to an inverse change in the quantity of ADH released from the gland. These conditions will be listed and the evidence considered for and against the hypothesis that the central nervous system is responsible for controlling the liberation of the ADH, i.e., that the hypothalamic hypophyseal tract is efferent. The conditions which bring about a change in the rate of excretion of water by the kidneys are as follows: 1, lesions of, or operative interference with, the posterior lobe, the nerve fibres passing to the pars nervosa, or the cells of origin of these fibres, 2, direct stimulation of nerve fibres connected with the pars nervosa, 3, exercise, 4, emotion, 5, conditioned stimuli and hypnotic suggestion, 6, certain sensory stimuli, 7, the administration of certain anaesthetic and narcotic drugs, 8, dehydration, 9, hydration.

1 *Lesions of, or operative interference with, the posterior lobe, the nerve fibres passing to the pars nervosa, or the cells of origin of these fibres*. Frank in 1910 (52) first drew attention to the clinical evidence involving the posterior lobe of the pituitary in the production of diabetes insipidus, and three years later Camus and Roussy (24) demonstrated that a lesion of the hypothalamus that did not include the hypophysis also led to the appearance of diabetes insipidus. Since at this time Cajal's observation on the innervation of the pars nervosa had not been properly grasped, these apparently contradictory results led to considerable confusion and argument. Later anatomical studies drew attention to the close link between the hypothalamus and hypophysis and suggested that there was evidence that the opposing hypotheses were in reality compatible.

There have been difficulties in the way of accepting the hypothalamic-hypo-

physcal route for the control of ADH production. It was found experimentally that removal of the posterior lobe alone or section of the stalk (99) (119) (159) did not necessarily result in more than a temporary disturbance of the water balance. Further, simple, or so-called total, hypophysectomy did not lead to the appearance of diabetes insipidus (12) (24) (46).

Fisher, Ingram and Ranson (51) showed that the neural division of the pituitary gland was composed of the pars nervosa, the stalk and the median eminence, and that in all these parts the minute structure was similar and pituicytes could be found. In different animals the anatomical arrangement of the parts varies, but it is evident that all three parts must be removed in order to bring about a complete loss of secreting tissue. This finding appears to dispose satisfactorily of the first of the above mentioned difficulties. The second difficulty, that diabetes insipidus is not evident after "total hypophysectomy" is explained by the observation first of Crowe, Cushing and Homans (34), and later of others, that the anterior lobe has a diuretic function, probably an indirect one (79) (148), and that in its absence a maximum polyuria is improbable or impossible. Once the anatomical arrangement of the posterior lobe tissue and the function of the anterior lobe were better understood most of the contradictions and difficulties were resolved.

Diabetes insipidus can follow posterior lobe removal provided enough of the gland is removed (51). In some species this is not easy. Cushing (35) succeeded in producing intense diabetes insipidus in a dog by the position of a silver clip on the pituitary stalk. Post mortem examination showed a loss of nerve fibres distal to the clip. Fisher, Ingram and Ranson (51) finally demonstrated conclusively that in cats and monkeys the normal activity of the pars nervosa depended on the integrity of its innervation. It has already been mentioned that they had observed that destruction of the supra-optic nuclei resulted in atrophy of the nerve tract to the pars nervosa, and that the pars nervosa itself also atrophied, becoming afibrillar and cellular. Diabetes insipidus resulted in all instances where these changes had taken place to a sufficient degree. These results have been confirmed by others for cats, dogs and monkeys (9) (43) (80) (148) (158). That following the degeneration of the supra-optic nuclei the changes seen in the pars nervosa are of the nature of atrophy is further shown by the fact that no antidiuretic substance can be extracted from such glands (73) (74) (50). Fisher, Ingram and Ranson (51) localised their lesions with great accuracy with the aid of the Horsley-Clarke stereotaxic instrument and showed that the fluid exchange was not affected by lesions in the preoptic area, the lateral and posterior hypothalamus, the mammillary bodies and the tuberal nuclei. In order that diabetes insipidus may be produced the supra-optic cells and tracts must be destroyed nearly completely and bilaterally. In monkeys the water exchange remains normal if 12-16 per cent (117) or 12-14 per cent (157) of supra-optic cells survive operation. The survival of only 5-7 per cent of supra-optic cells is associated with the existence of a marked polyuria (117). In dogs diabetes insipidus is seen provided 85 per cent, or more, of supra-optic cells have atrophied (81) (148). That the survival of such a small part of the hypothalamic-hypophy-

seal system is sufficient to maintain a normal water exchange is not surprising if it be remembered how minute are the doses of posterior lobe extract that can inhibit water diuresis (75) (146) (179)

The literature on the pathological findings in cases of diabetes insipidus in man is enormous and varied, as any lesion of any type encroaching on the hypothalamus or pituitary may lead to the appearance of diabetes insipidus, or it may not. The difficulty here as with all clinical material is that only rarely is the lesion sharply and accurately localised. Furthermore, until recently, the degenerative changes that can occur in a macroscopically normal posterior lobe were not appreciated and therefore not looked for. The same is true of the degenerative changes in the supra-optic and paraventricular nuclei. Many observers believed diabetes insipidus to be purely hypothalamic in origin since it often followed an infection of the meninges which could not directly affect the pars nervosa. For example, Lhermitte (111) (112) describes how diabetes insipidus may result from syphilitic basal meningitis in which at autopsy degenerative changes could be found in the hypothalamic nuclei whilst the posterior lobe appeared to be normal. Others (62) (65) believed that destruction of the pars nervosa, as by metastases, was the cause of diabetes insipidus. Others again (47) (48) (100) believed that the hypothalamus and hypophysis must be considered a single functional unit. Perhaps the most carefully observed and striking cases in favour of the hypothesis of the neuronal control of the posterior lobe secretions are those described by Biggart (6) (7) (8). He found that diabetes insipidus could follow complete destruction of the pars nervosa or interruption of the stalk with resultant loss of cells in the supra optic and paraventricular nuclei, and also followed lesions of the hypothalamus involving the supra optic nuclei with secondary atrophy of the pars nervosa. In one case of 30 years' duration the histological appearance of the shrunken and atrophied pars nervosa and the marked loss of supra optic cells was in every way comparable with the findings of Fisher, Ingram and Ranson (51) after experimental destruction of the supra-optic nuclei in cats and monkeys. In their case Davison and Selby (38) describe similar autopsy findings.

In man, as in animals, diabetes insipidus may follow stalk section. Dandy (37) reported the case of a young woman whose pituitary stalk was sectioned at operation and who promptly developed a permanent diabetes insipidus. As this case was still alive at the time of writing (11 years after operation) it was not known what percentage of supraoptic cells had atrophied. In a man suffering from malignant hypertension the pituitary stalk was divided. This man did not develop diabetes insipidus. Five months after operation he died and at the autopsy it was found that about 15 per cent of his supra-optic cells appeared to be normal (157). This suggests that in man, as in monkeys, the survival of a small proportion of the total number of supra-optic cells, and presumably, therefore, of a similar proportion of pars nervosa tissue, is sufficient to prevent the onset of diabetes insipidus.

That there can be a polyuria of central origin associated with atrophy of the pars nervosa which is not controlled by the administration of posterior lobe ex-

tract (6) (86) (87) (160) (174) is no disproof of the hypothesis that the hypothalamus controls the release of the antidiuretic hormone. The type of diabetes insipidus resistant to posterior lobe extract has yet to be produced experimentally and analysed. The origin of the resistance to antidiuresis is unknown. It may be due to some factor which counteracts the action of posterior lobe extract. Such a factor might be a central nervous irritation disturbing the renal circulation. In the cases described by Snell and Rowntree (174) and Roehm (160) there was an obvious disturbance of the peripheral circulation, and in one of the cases reported by Hoff and Wermer (86) posterior lobe extract became effective when the cerebral pressure was reduced.

It may be seen that observations of the type described in this section offer considerable evidence for the belief that the release of the ADH and the functional existence of the pars nervosa depend upon the integrity of the nerve connection between the gland and the supra-optic nuclei and that when this connection is severed the pars nervosa atrophies and its loss of function is made evident by the appearance of polyuria.

2 *Direct stimulation of nerve fibres connected with the pars nervosa* It has been reported (27) that on stimulating the central end of the vagus to the isolated viviperfused head of a dog there was an increase in the rate of urine flow from the kidneys of the perfusing body. The urine collected during stimulation was tested on rats and was found to contain an antidiuretic substance which was absent on repetition of the experiment after hypophysectomy. It was concluded that these observations demonstrated the release of the ADH from the pars nervosa on stimulation of the central end of the vagus. The response fatigued but showed recovery if the time interval between stimuli was lengthened (29). This was correlated with exhaustion of the secretory granules in the pituicytes. The functional significance of this reflex is obscure, as is the site of the peripheral sensory receptors.

A slight increase in the rate of urine flow on stimulating the superior cervical ganglion of cats anaesthetised with ether and urethane has been described (168). As this increased rate of urine flow was observed after high cord section it was believed to be of pituitary origin.

Evidence that the pars nervosa is under the influence of the central nervous system has been offered by Karplus and Pecznak (97) and Haterius and Ferguson (78). The former stimulated the hypothalamus in etherised cats and reported that they could thereafter detect the oxytocic and melanophore hormones in the cerebrospinal fluid and in the blood. These results need confirmation. Haterius and Ferguson stimulated the stalk region and noted a resultant increase in uterine activity similar to that produced by the injection of pitocin and unlike the response of the uterus to adrenalin.

There is evidence that stimulation of the stalk can bring about the release of ADH also. Ingram and Barris (91) found that stimulation of the junction of the pars anterior and pars tuberalis resulted in etherised cats in a diuresis. Haterius (77) also applied direct electrical stimulation to the pituitary stalk. He used rabbits under light chloralose and urethane anaesthesia and noticed

that the stimulation led to an inhibition of a pre-existing water diuresis. No inhibition appeared if 2-4 days earlier the pituitary stalk had been transected between the gland and the point of application of the stimulus. Using a different approach Boyd, Lee and Stevens (13) describe what they believe to be a reflex liberation of ADH on stimulating with flashes of light the eyes of unanaesthetised rats. This stimulation caused a diuresis in the rats and did not inhibit an already existing water diuresis. They believed that the diuretic effect depended on the amount of hormone liberated, as on injecting posterior lobe extract in doses equivalent to the amount found in a single rat pituitary gland they obtained similar results. Smaller doses of posterior lobe extract had an antidiuretic effect.

Obviously it is difficult to perform experiments of this type under normal conditions, but, allowing for this limitation, the results do offer evidence for, and not against, the neural control of ADH production.

3 and 4 *Exercise and emotion* Mackeith, Pembrey, Spurrell, Warner and Westlake (115) noticed that exercise inhibited water diuresis and similar results were obtained by others (41) (101) (193) (195). This inhibition occurs as much in the denervated as in the innervated kidney (101) and persists after the division of both splanchnic nerves (101). It is unlikely, therefore, that it can be due to renal vasomotor changes of central origin or to the liberation of adrenalin. The similarity of the time course of the inhibition due to exercise and that due to injection of posterior lobe extracts has been described (101) and it was suggested that the ADH was responsible for the inhibition due to exercise. That the magnitude of the inhibition due to exercise appeared to be independent of the amount of work done was noticed by all the observers mentioned. In one of Dobreff's experiments it may be seen that in prolonged exercise there was an early and profound inhibition of diuresis, but that recovery set in whilst the exercise was still proceeding (41). In the experiments of Mackeith et al. it was shown that the inhibition appeared early during prolonged exercise and, therefore, could not be due to increasing loss of water through the skin and lungs. The variability of the inhibitory effect of exercise on the excretion of water suggested to Rydin and Verney (164) that the cause must lie elsewhere than in the exercise itself, and in view of observations that emotion similarly inhibited diuresis they set out to find whether this was the real origin of the inhibition of exercise.

Bernard (5) was the first to draw attention to the fact that emotion could inhibit the flow of urine. He ascribed this inhibition to renal vasomotor changes, but any possible participation of the renal nerves in the inhibition has been excluded by showing that it can occur in the dog after the separation of all organic connection between the animal and its kidneys (180). It seemed, then, that a humoral agent must be responsible for the effect observed. The time course of the inhibition resembled that due to the administration of appropriate doses of posterior lobe extract.

Using dogs exercised on a controlled running platform Rydin and Verney (164) showed that mild exercise begun during the height of water diuresis pro-

duced within two minutes a prolonged fall in the rate of urine flow, but that if the dogs were repeatedly exercised under similar conditions the inhibitory response diminished and was finally extinguished. If at this stage of acclimatisation an emotional stimulus, in this case a cacophonous noise, was simultaneously introduced the inhibition reappeared. They further observed that the time relationships of the inhibition were the same whether it were initiated by exercise or emotion. They then applied only an emotional stimulus during water diuresis and once again observed the same type of inhibition. They felt that these observations justified the belief that mild exercise depended on its emotional content for any inhibitory effect seen. The course of the antidiuretic response to emotional stress was unaltered by successive operations comprising the division of the renal nerves, removal of the right suprarenal gland and denervation of the left, and decentralisation of the whole abdominal sympathetic system with removal of ganglia L2-S1 inclusive. Nor did the inhibition of the urine flow show any parallelism with blood pressure changes simultaneously recorded. When blood pressure changes local to the kidney were induced the urine flow followed these closely. Lastly they showed that the inhibition of urine flow following the injection of appropriate doses of adrenaline were sharp and fleeting and in no way resembled that due to exercise, emotion or posterior lobe extract. This evidence all pointed to the existence of a common cause for the inhibition of water diuresis due to exercise and emotion and indicated that this common cause was not adrenaline.

O'Connor and Verney (143) offered more direct evidence that the posterior lobe of the pituitary is concerned in the emotional inhibition of urine flow. They found that in dogs after removal of the posterior lobe the inhibitory response to an emotional stimulus was either greatly reduced or abolished. Since they were removing the posterior lobe alone it was to be expected (51) that some antidiuretic activity should still be present. O'Connor and Verney determined the quantity of posterior lobe extract that had to be injected in order to produce the degree of emotional inhibition observed both before and after operation. In one dog before operation 5-10 millunits posterior lobe extract gave the same degree of inhibition as the emotional stimulus. After operation the average dose required was only 0.2 millunit. There was apparently a functional residuum of 2-4 per cent of the total posterior lobe in this dog.

In a few unpublished observations Pickford attempted the assay of the ADH content of dogs' urine collected during the inhibition caused by a just resented electrical stimulation applied to the flank. The urine was concentrated by the method described by Gilman and Goodman (63) and assayed by Burn's (19) rat method. The pooled urine from four periods of stimulation inhibited diuresis in the rats to about the same extent as did 5 millunits of posterior lobe extract. After removal of the posterior lobe from the dogs pooled urine from six periods of stimulation had no inhibitory effect on water diuresis in the rats.

Emotional stress can give rise to a polyuric condition resembling diabetes insipidus, but different in that it is curable by suggestion (120).

The emotional inhibition of water diuresis can be mediated by other agents

than the ADH (72) Such inhibitions persist after hypophysectomy or stalk section, are accompanied by a decrease in the renal creatinine clearance and are seen only in certain individuals. These observations do not invalidate the conclusions to be drawn from the experiments described in this section, namely, that emotional stimuli bring about a release or retention of the ADH. We do not know whether the central nervous stimulation is conveyed direct to the pars nervosa or whether changes are first induced in the water balance of the tissues and blood.

*5 Conditioned stimuli and hypnotic suggestion* It has been shown that an appropriate stimulus of the central nervous system can induce a water diuresis in the absence of the ingestion of water (88) (108) (123). In one series of experiments it was suggested to patients under hypnosis that they had had a drink of water and they were given an empty glass to hold. Shortly after recovery from hypnosis the rate of urine flow increased, the form and time course of this increase being similar to those in control experiments in which water had been ingested without hypnosis. There was a reduction in the specific gravity of the urine and in the concentration of the blood (123). It has also been described how in one dog out of the four that were in use at the time water diuresis appeared in response to the conditioned stimulus of a musical note (126).

Others (4) (21) (22) (66) (69) have recorded similar findings, using as the conditioned stimulus the introduction of the rectal catheter by which the water was usually administered, or the mere placing of the dog in the position and environment in which the unconditioned stimulus was repeatedly given. This diuresis and its inhibition on the approach of a cat are, in part at least, of hormonal origin since they were present to an equal degree in both kidneys even after the denervation of one kidney.

There is no information as to the means whereby this conditioned diuresis is mediated, whether the higher centres, using the nerve paths which undoubtedly exist, induce a direct inhibition of pars nervosa activity, or whether the effect is first on the tissues and blood and only secondarily on the pars nervosa.

Certain observations show that it is possible to set up a conditioned inhibition of an established water diuresis (42) (179). It was found, as will be described later, that in the dog inhibition of diuresis resulted from the insertion and movement in the lumbar area of a needle. It also became evident that this inhibition of water diuresis was more readily caused in animals in which the "operation" had been performed more than once. In one dog it was found possible to cause a diminution of the rate of urine flow by making ostentatious preparations for performing the puncture.

*6 Sensory stimuli* Certain sensory stimuli are effective inhibitors of water diuresis (179). It was found that in dogs the movement for a few minutes of a needle in the lumbar area resulted in a complete inhibition of diuresis lasting for about 40 minutes. This was the case even when the area of skin and subcutaneous tissue had previously been infiltrated with 2 per cent novocaine. On two occasions the mere shaving of the back over the lumbar region was sufficient to inhibit diuresis. Shaving hair from the leg prior to an intravenous injection



was never observed to lead to an inhibition. The question remains open whether stimulation of the lumbar region is more likely to result in an inhibition than a similar stimulus applied elsewhere.

By denervating one kidney and using the other as a control Theobald and Verney (180) showed that there is no essential difference in the response of the two to the inhibitory influence of afferent nerve stimuli on an established diuresis. That this phenomenon finds its cause in the ADH of the pars nervosa is suggested by the comparison of the inhibitions due to afferent stimuli and to posterior lobe extract, both show the same time lag in onset, the same time course, and after both albumin may be found in the urine.

That the hypothesis of a pituitary cause of the inhibition of diuresis due to afferent stimuli is justifiable has been shown more directly by Haterius (76) (77) who used rabbits under light chloralose urethane anaesthesia and found that in them, as in dogs, stimulation of the lumbar area led to the inhibition of an established water diuresis, but that 2 to 4 days after the destruction of the pituitary stalk lumbar stimulation in no way interfered with the normal rise and fall of renal water loss in diuresis. Further, in 5 out of 8 rabbits stimulation of the pituitary stalk inhibited diuresis, whilst a similar stimulus applied a few days after destruction of the stalk resulted in no inhibition, thus demonstrating that the nerve paths in the stalk must be intact for the antidiuretic action to be apparent, i.e., for the ADH to be liberated in response to nerve stimulation.

7 *The administration of certain anaesthetic and narcotic drugs.* Under the influence of a large number of anaesthetics and narcotics the diuretic effect of water is diminished or abolished (44) (53) (54) (64) (139) (152) (173) (192), though some workers report that drugs such as chloralose and paraldehyde increase the response to water (11) (105) (137) (183). This has not been confirmed by others (44) (179). Bourquin (12) reported that morphine in small doses increased, and in larger doses diminished the renal response to water. The depressant effect of narcotics on water diuresis is probably not a direct action on the kidneys (44), it does not run parallel with that on consciousness (11) (44) (54) (125) or thirst (44), nor is the integrity of the renal or splanchnic nerves essential (53) (114) (183). In anaesthesia there may be a delay in water absorption (84) but that this is by no means the only cause of a reduction in the diuretic response is shown by the fact that the diuresis induced by intravenous or intraperitoneal water (53) (141) is also affected by anaesthetics. The inhibitory effect of these drugs is not due simply to their blood concentrating action (17) (44). It appears, then, that the inhibitory effect of anaesthetics on diuresis must be, in part at least, of hormonal nature. Molitor and Pick (134) (135) (136) (137) postulated the existence of a water controlling centre in the midbrain. This centre they conceived to be under the inhibiting control of the cerebral cortex, and if this restraining influence were removed either surgically or by optimal doses of narcotics such as paraldehyde, the centre would become overactive and water diuresis would be increased, if the centre were depressed then so, also, would be water diuresis. This hypothesis was based almost entirely on experiments on the antidiuretic action of posterior lobe extracts under various conditions. As

proof of the overaction of the water centre under the influence of what they considered a purely cerebral cortical narcotic they reported that paraldehyde rendered null the action of posterior lobe extracts. They believed the posterior lobe extract to act not on the kidneys but on the tissues, either directly, or through the water centre. Their results could not be repeated, in fact, the reverse was found to occur (179). If narcotics by their direct or remote action on a water centre are supposed to counteract the effect of posterior lobe extract at that site, it is difficult to explain how the extract can be an effective antidiuretic in decorticate and decerebrate animals (45) (94) and in anaesthesia (105) (139) (179). If the explanation offered be that the posterior lobe hormone acts on the tissues, then why, in anaesthesia, does blood dilution not result in a diuresis (17) (45) (172)? Further, how does fluid become available for excessive salivation during the inhibition produced by posterior lobe extract, both in the narcotised (179), and in the normal (148) dog, and how can the head of one dog inhibit the urine flow in the isolated perfused kidney of another (187)? The conclusion is inevitable that there is no water centre in the sense postulated by Molitor and Pick, that posterior lobe extract has a direct action on the kidneys and that in all probability the inhibition of water diuresis seen under anaesthesia is, in part at least, due to the release of increased quantities of ADH. This does not exclude the participation of other factors under certain as yet ill-understood conditions. Thus, in the case of diabetes insipidus described by Frey and Kumpless (54) the administration of chloral hydrate, trional or morphine reduced the polyuria. Whether there was any surviving normal pars nervosa tissue is unknown. The transitory polyuria of experimentally induced diabetes insipidus begins whilst the animal is still deeply anaesthetised (148). As yet the cause of the transitory phase is unknown. Moreover here as in the previous case, the renal nerves were intact, and the kidney activity may have been modified by centrally initiated vasomotor changes. Bodo and Sweet (10) report that the percental fall in water excretion under morphine and phenobarbital is the same in normal, completely hypophysectomised and in adrenal inactivated liver denervated dogs, and believe, therefore, that neither the pituitary gland nor the adrenals can be involved in the antidiuresis. Here too, the kidneys were not denervated and there is no mention of histological control of the "total" hypophysectomy.

Bruger, Bourne and Dreyer (17) believe that in avertin anaesthesia, despite blood dilution, a fall in blood pressure may be sufficient to account for the antidiuretic action.

Koiwa (102) reports that picrotoxin and  $\beta$  naphthylamine despite their known central stimulant action have an antidiuretic effect, which he believed depended on a renal vasomotor effect.

It may be concluded that the anaesthetics and narcotics by their action in modifying the balance of activity in the nuclei of the central nervous system bring about, through nerve channels, an increased release of ADH, thus accounting for the antidiuretic action observed. This does not exclude the possibility that the kidney is also directly influenced through its nerve supply.

8 *Dehydration* Since the posterior lobe of the pituitary produces an antidiuretic hormone it is a reasonable working assumption that in conditions demanding the conservation of water a greater than normal quantity of ADH should be released. If the conditions be sufficiently stringent it should be possible to show that the gland itself suffers depletion or that fluids likely to carry or receive the hormone contain an increased concentration of it, in other words, dehydration whether due to restricted intake or excessive output should be a strong stimulus to the release of ADH. A number of experiments support this hypothesis.

Using the direct method of assaying the antidiuretic content of the posterior lobe Simon (169) and Simon and Kardos (170) found that in rabbits, guinea pigs and cats after 4-5 days on a dry diet the antidiuretic activity of the posterior lobes was very low compared with controls having an ample supply of water. A decrease in food intake was not responsible for the change since the posterior lobes of guinea pigs and rabbits which had had water ad libitum and no food showed a normal antidiuretic activity. Fighting, work and electrical stimulation had no effect on the hormone content of the gland. Similar findings are reported in rats and dogs (73) (74) (106).

The number and size of the cells in the pars nervosa have been found to vary with the water intake. In rats on a dry diet the cells increased in size and number, if after a period of dry diet water was allowed ad libitum even for one day the pars nervosa was found to present a normal appearance (59) (60).

In searching for the ADH in body fluids certain difficulties are encountered. First, that as we do not know the chemical nature of the principle assays must be biological. The methods of assay are based on the fact that posterior lobe extracts inhibit an induced water diuresis and the polyuria of diabetes insipidus. Dogs (138) (143) (145), mice (61) (140), and rats (19) have been used as test animals. The rat method of Burn (19) or a modification of it is that most commonly used. The second difficulty is that in the higher mammals the kidney is sensitive to minute amounts of the hormone (75) (101) (146) (165) (175) (179), consequently under normal conditions it is probable that the quantities present in the body fluids will be difficult of detection. Thirdly, the body can rapidly inactivate administered posterior lobe extracts (85) (95) and, probably also, therefore the normally released hormone. However, it has been shown (95) that, in terms of the pressor fraction, about 30 per cent of injected posterior lobe extract can be recovered in the urine where it is relatively stable. Using this fact, and starting with the assumption that the ADH is an important factor in the regulation of the volume of the body water Gilman and Goodman (63) argued that following dehydration the level of the ADH in the blood should reach a concentration sufficiently high to allow it to be excreted in detectable amounts in the urine. In support of this argument they found that the urine of rats subjected to dehydration contained appreciable amounts of ADH and that this urinary antidiuretic substance seemed, from its chemical properties, to be identical with that which can be extracted from the posterior lobe. They compared the antidiuretic activity of the urine of normal freely hydrated rats, normal rats

dehydrated either by deprivation of water or the administration of hypertonic NaCl, and similarly treated hypophysectomised rats. There was no detectable antidiuretic activity in the urine of the hydrated animals. Dehydrated normal rats during the time of collection excreted the equivalent of approximately 100-200 millunits of antidiuretic substance. Hypophysectomised rats' urine, despite the fact that in the time allowed these animals excreted more urine and were therefore more dehydrated than normals, contained no ADH. These results have been confirmed for rats (14) (122) and dogs (74).

Ingram, Ladd and Benbow (92) (93) found that there was an appreciable quantity of ADH in the urine of normal dehydrated cats but that none could be found in the urine of cats, whether hydrated or dehydrated, made diabetic by the interruption of the supra-optic tracts. They further showed that the kidney of a diabetic animal could excrete injected posterior lobe extract, thus ruling out the possibility that no active substance can be found in the urine because the kidney has become incapable of excreting it.

Marx (124) searched the blood for the presence of the ADH and believed that he had demonstrated that the blood of thirsting dogs contained an antidiuretic substance which was absent from the blood of hydrated dogs during diuresis. Marx and Schneider (127) reported the presence in the blood of normal men, but not in the blood of a case of diabetes insipidus, of an antidiuretic substance. Melville (128) found an antidiuretic substance in normal human and dog blood, but did not feel justified in identifying it positively with the hormone of the posterior lobe. The work of Marx has been criticised (188) on the grounds that the circulation in the blood of the described amounts of ADH would, considering the great sensitivity of the human and dog kidney, result in a permanent inhibition of urine flow. There is a possible explanation of the relatively large amounts of ADH found by Marx (82) (109). It has been shown that posterior lobe extracts after addition to rabbits' blood will not pass through an ultrafilter, i.e., the hormone appears to suffer adsorption by one or more of the blood colloids. It has been suggested that the ADH is held in the blood in an inactive state, to be released on reaching the renal tubules. This certainly might explain the presence in the blood of the hormone in amounts greater than the minimum inhibitory dose, but leaves open the method whereby only that amount of the hormone appropriate to the circumstances is released in the kidney.

That there are possible sources of antidiuretic substance other than the posterior lobe has been shown (1) (167) (181). This fact is intrinsically interesting and shows that caution must be used in identifying any particular antidiuretic substance with that derivable from the posterior lobe, but it does not invalidate the conclusions to be drawn from those experiments where antidiuretic substance was absent from the urine after, though present before, hypophysectomy. Walker (191) makes the criticism that hypophysectomy, by a secondary effect on other endocrines, may disturb the production or release of those antidiuretic substances whose origin is elsewhere than from the pituitary. This criticism is rendered invalid by the observation that the antidiuretic substance disappears from the urine after interruption of the supra-optic tracts (93), an operation

which, as far as is known, does not have an effect on any gland except the pars nervosa

The foregoing experiments give ground for believing that dehydration is a stimulus to the production and release of the ADH of the pars nervosa and that this stimulus is transmitted through the supra-optico-hypophyseal nerves

9 *Hydration* If dehydration be a powerful stimulus for the release of the ADH with the object of conserving water, then hydration may be expected to inhibit the release of the hormone. Thus, on Verney's hypothesis (101) (188) an acute water diuresis is due to a temporary reduction in the amount of ADH liberated, and the delay in time between the peak changes in the water load and in the renal excretion rate is related to the speed of destruction of existing ADH and of depression of pars nervosa activity. If this be the case, then after loss of pars nervosa tissue sufficient to lead to diabetes insipidus the maximum urine flow rate might be expected to be quicker in onset and greater in degree. Kłisiewicz et al (101) give figures in a normal and a diabetic man of the period in minutes between the midtime of drinking and the first increase in the rate of urine flow. In the diabetic even when thirst was present, which must be supposed to mean that there was still some degree of dehydration, this period was shorter than in the control. In a dog which had had the whole pituitary gland removed and the supra-optic tracts sectioned and in which at post mortem only about 2 per cent of surviving supra-optic cells were found, the rate of urine flow in diuresis was consistently higher after as compared with before operation (148). That in experimental diabetes insipidus the maximum rate of urine flow in water diuresis is frequently no higher than in normal animals does not invalidate the pituitary hypothesis, since such minute quantities of posterior lobe extract are effective in altering the rate of urine flow, that even a small amount of surviving pars nervosa tissue may be sufficient to ensure a normal water diuresis (75) (179).

Fee (45) and Newton and Smirk (142) from their observations came to the conclusion that neither the hypothalamus nor the pars nervosa was of primary importance in the production of polyuria and water diuresis.

They based this belief on the results of their observations on the water excreting capacity of decerebrate and totally hypophysectomised cats and dogs. They found that, allowing time for the excretion of the volatile anaesthetic used in the preparatory stages of the experiments, such animals could excrete ingested water in an apparently normal manner, there was the normal interval of time between the absorption of the water and the onset of diuresis, the maximum rate of urine flow occurred at about 80 min after ingestion ended, and thereafter the urine flow rate fell away in a normal manner. They failed to observe a true persistent polyuria. Their results were only in part confirmed by Tsai (182).

A possible explanation of these results is afforded by an examination of the figures Fee gives (45). Using dogs he administered as much as 350 cc water and the maximum urine flow rate attained was 22.3 cc per 20 min. After 3 hours, of the 350 cc given, 122 cc had been excreted. This low rate and small total excretion can by no means be considered typical of a normal water diuresis. The weight of the dogs is not given, but experience with unanaesthetised dogs of

about 12 kgm shows that after 300 cc of orally administered water a maximum urine flow rate of at least 60 cc per 20 min may be expected, and that in 3 hours about 85 per cent, or more, of the volume given has been excreted by the kidneys. On the other hand, after removal of the whole pituitary gland an unanaesthetised dog has, after water administration, a maximum urine flow rate that is about half, or less, of the preoperative rate under similar conditions (148) and does not excrete nearly as much as usual in 3 hours. Thus, in these dogs an increase in the water load does result in an increase in the rate of urinary water excretion, but this increase is far below the normal and may persist for a longer time. If a polyuria be present it is small in degree. The poor response of the kidney to water appears to be due to the loss of the anterior lobe of the pituitary. Thus, the diuresis obtained by Fee and Newton and Smirk, whose animals had no anterior lobe, may have been of the type seen in unanaesthetised totally hypophysectomised dogs, where the loss of posterior lobe tissue tends to polyuria and increased diuresis, but the loss of anterior lobe tissue counteracts these (79) (148). Nor is it justifiable to assume, as Newton and Smirk did, that the capacity of the kidneys to exhibit a water diuresis is evidence of their capacity to exhibit a spontaneous polyuria. These two capacities can vary independently of each other. An unanaesthetised dog, at a time early after operation, when it is polyuric, may be incapable of producing a normal water diuresis (148). The observations of Fee and Newton and Smirk, then, are no disproof of the existence, under normal conditions, of a decreased liberation of ADH in response to the ingestion of water. Moreover, it has never been suggested that, in any given conditions, the ADH is the only factor limiting the rate of urine flow.

Hart and Verney (75) found that the fall in the rate of urine flow produced by pituitary extracts in the course of a maximum water diuresis was smaller when the extract was given early than when given late. Pickford (146) in a series of experiments planned to discover whether a simple relationship existed between the degree of inhibition in the rate of urine flow resulting from the administration of pituitary extract on the one hand, and the amount of tissue water in excess of the normal on the other (the water load), found that there was a roughly inverse proportionality between the two. Thus, when as little as 0.00002 cc (0.2 millunit) posterior lobe extract was injected intravenously into dogs early during the course of a water diuresis the resultant inhibition was considerably less than when the same dose was given later in diuresis. This difference in response to the extract may easily be explained if it be assumed that with high water loads the production or release of the naturally occurring hormone is inhibited. At such a time an administered dose of extract would be added to a small quantity, or none, of the spontaneously produced hormone and would be, therefore, relatively ineffective. As the water load falls increasing quantities of the natural agent would be present in the blood and the injection of exogenous hormone would result in a greater effective concentration and, hence, of a greater degree of inhibition than previously.

It would appear that when the water load of the body is high, the liberation of the ADH from the pars nervosa temporarily ceases until such time as the

water load falls to normal levels When the body is dehydrated there is an increased circulation of ADH in order that water may be retained by the kidneys and the osmotic balance of the blood and tissues preserved In dehydration the posterior lobe activity probably varies in response to nerve impulses Until the contrary be proved, it would be needlessly complex to suppose that whilst the posterior lobe is stimulated by a neuronal mechanism it is inhibited by some other means Moreover, in all the previous sections it has been shown that the balance of evidence is in favour of the regulation of the antidiuretic activity through the hypothalamic-hypophyseal tracts

It remains to be considered by what means the nerve impulses are transmitted

*Transmission of the stimulus* It is probable that the transmission of the stimulus to the posterior lobe depends on the release of acetylcholine at the neuronal or neuro-glandular junction It has been shown that acetylcholine injected into the ventricles of cats produces effects similar to stimulation of those parts (39) (40) Others have described how on stimulation of the central end of the vagus a cholinergic substance, probably acetylcholine, was released from the central nervous system This substance was believed to originate in the hypothalamus (25) (26) During the time of vagal stimulation a pressor and an antidiuretic substance were described as being present in the jugular blood, provided that the hypophysis was present (28), i.e., acetylcholine liberated in the hypothalamus by stimulation of the vagus caused the release of posterior lobe hormones into the circulation Pickford (147) showed that in the normal conscious atropinised dog intravenous acetylcholine inhibited a water diuresis Blood pressure records taken before and during the response to acetylcholine showed that it was improbable that the prompt and brief blood pressure changes could be concerned in the delayed and prolonged inhibition of urine flow Nor could adrenaline be the causative agent as this substance given intravenously produces a sharp, transient and synchronous change in the blood pressure and the urine flow Further, others have shown (164) (166) (189) that vasomotor changes localised to the kidneys bring about changes in the rate of urine flow whose time course closely follows that of the vascular changes Denervation of the kidneys did not abolish the response to acetylcholine As the time course of the inhibition and the changes in chloride concentration of the urine were similar to those seen after administration of posterior lobe extract, and since acetylcholine, like posterior lobe extract produced a diuresis in the thirsting unanaesthetised dog, it seemed probable that the inhibitions were causally related If indeed that were so, then loss of the pars nervosa should abolish the response to acetylcholine This was found to be the case After removal of the posterior lobe neither the antidiuretic nor the diuretic effects of acetylcholine could be elicited, nor the rise in the rate of chloride excretion

A few observations (149) both before and after removal of the posterior lobe have been made on the antidiuretic content of the urine excreted during the inhibition of diuresis produced by acetylcholine The methods of Gilman and Goodman (63) and Burn (19) were used It was found that before operation the pooled urine from 4-6 periods of acetylcholine administration resulted in an

inhibition of diuresis in the rats, whereas after removal of the posterior lobe similarly collected urine did not inhibit the rate of excretion in the rats. These findings all point to acetylcholine as the transmitter of the impulse to the pars nervosa, but give no information as to its site of action, whether at the hypothalamic synapses or at the nerve terminations in the posterior lobe.

### CONCLUSIONS

The release into the circulation of the ADH made in the pars nervosa appears to be under the control of the central nervous system. The impulses travel to the pars nervosa along the hypothalamic hypophyseal tracts, and somewhere on this route depend on acetylcholine for their transmission. The primary stimulus which initiates this reflex is not known with certainty. The receptors for this stimulus are central and not peripheral.

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# PRESENT VIEWS ON THE MODE OF ACTION OF ACETYLCHOLINE IN THE CENTRAL NERVOUS SYSTEM

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The conception that acetylcholine acts as chemical transmitter in the central nervous system is a logical extension of the theory of chemical transmission by acetylcholine across ganglionic synapses and from motor nerve endings to motor endplates of skeletal muscles. About ten years ago Dale (1934), in discussing the evidence for the transmission by acetylcholine across ganglionic synapses, pointed out that this theory involved a much wider aspect, namely, similar transmission at central synapses. A few years later, in his Huxley Lectures (1936/37), he took up the problem again. He drew attention to the fact that Eccles had used the sympathetic ganglion "as furnishing an acceptable model of the synapses of the central grey matter" and that Sherrington had looked upon the transmission at a motor endplate "as probably furnishing a pattern, or paradigm, of what happened at a central synapse". It is thus not surprising that the methods of investigation used for the ganglion and voluntary muscle should be extended to the central nervous system.

The following three lines of research have supplied the main evidence on which the acetylcholine theory in the peripheral nervous system is based: (1) a study of the effects of acetylcholine and of drugs which, like atropine or nicotine, influence the response to acetylcholine, (2) a study of the effects of eserine and other inhibitors of cholinesterase on the response to nervous impulses and (3) experiments which show that acetylcholine is in fact liberated during nervous activity. Supporting evidence has been obtained from determinations of acetylcholine and cholinesterase contents of a tissue under different conditions and from the presence in nervous tissue of an enzyme system responsible for the synthesis of acetylcholine. In the last ten years these lines of research have been applied to the central nervous system and the results thereby obtained are reviewed in the following sections.

1 CENTRAL ACTIONS OF ACETYLCHOLINE. The peripheral effects of acetylcholine have been classified as muscarine- and nicotine-like actions (Dale, 1914). Effects on smooth muscles, heart and gland cells, like the similar effects of muscarine, are sensitive to atropine, whereas the effects on autonomic ganglia and medullary cells of the suprarenals and the motor endplates of mammalian skeletal muscles are not affected by atropine, or at least not in doses which affect muscarine-like actions, but they are abolished by large paralysing doses of nicotine.

Acetylcholine has both stimulating and depressant actions on the central nervous system and both actions appear to be sensitive to atropine. They can therefore be classed as muscarine actions. It is, however, not advisable to make the atropine sensitivity the sole test for classification, its too strict application would separate even peripheral actions which belong together. For

stance, atropine abolishes the effect of acetylcholine on motor endplates in frogs but not in mammals and in larger doses atropine may depress the nicotine action of acetylcholine in other structures (Feldberg, Minz and Taudamara, 1934, Feldberg and Vartanian, 1935, Marrazzi, 1939) These limitations do not invalidate the atropine test, but in applying it we have to realize that very large doses of atropine depress cells in an unspecific way so that drugs other than the parasympathomimetic ones become ineffective. The central actions of acetylcholine differ from those of muscarine and other parasympathomimetic drugs. Pilocarpine, for instance, stimulates vegetative centres (Cushing, 1931) but, unlike acetylcholine, is ineffective when applied to the cerebral cortex (Miller, Stavratsky and Woonton, 1940).

The central actions of acetylcholine are best viewed together with those on motor endplates and on autonomic ganglia. They have this in common: the changes brought about at nerve cells or endplates initiate a wave of excitation propagated along a nerve or muscle fibre. If the acetylcholine persists at the nerve cell or endplate beyond the refractory period of the fibre a new wave of excitation may be initiated and this may be repeated over and over again. Thus the arterial injection of acetylcholine into a muscle causes a tetanic contraction of the muscle fibres, into a ganglion a repetitive firing along the postganglionic fibres. Similar waves of excitation will be initiated from the cells of the central nervous system.

Acetylcholine probably acts in these structures by virtue of its depolarising property which has been demonstrated by Boutner and Barnes (1941, 1942). They found that acetylcholine in minute doses produced electrical negativity when allowed to act on artificial interfaces of water and water insoluble substances resembling lipoids. A depolarisation of the endplate or nerve cell by acetylcholine might thus constitute the effective stimulus for the initiation of a wave of excitation. In the electric organ of some fishes, which consists of modified motor endplates lacking the contractile muscle fibres, this depolarisation is the final event and the electrical discharge may be looked upon as the sudden depolarisation of the plates, brought about by the release of acetylcholine from the nerve endings covering their ventral surfaces. In fact acetylcholine injected into such an organ has an electrogenic effect (Feldberg and Fessard, 1942).

When acetylcholine is released in the course of normal nervous activity, it is quickly inactivated, and consequently the cell surface remains depolarised for an extremely short interval, then becomes polarised and ready for the acetylcholine released by the next nerve impulse. But when acetylcholine is applied artificially or released in the presence of eserine, it will persist at the cell surface and the mechanism of depolarisation and polarisation may become deranged. This derangement may manifest itself as a depression of excitability produced by acetylcholine. The fact that under these conditions acetylcholine is predominantly depressant in action might thus find an easy explanation. Against this attractive theory, however, the fact must be stated that atropine antagonises the central depressant actions of acetylcholine and that there is no evidence that atropine is able to counteract, by a polarising action, the depolarising one of

**acetylcholine** The conception of acetylcholine as a depolarising agent is useful if applied to the theory of central transmission, but we have to realize that the mode of action of acetylcholine in the central nervous system is as little understood as that of any other drug acting peripherally or centrally

**Cerebral cortex** The effects of acetylcholine on the electroencephalogram were first recorded by Sjöstrand (1937) Acetylcholine applied locally to the cortex increased the amplitude of the large diphasic waves produced by strychnine or eserine and caused an increase in the frequency and grouping of the waves Strong concentrations or repeated applications of acetylcholine completely inhibited the waves Bonnet and Bremer (1937b) and Bremer (1938) divided the brain stem in cats above the pons in order to avoid the use of anesthetics and to prevent incoming impulses, under these conditions, injections into the carotid artery of 0.1 to 0.2  $\mu$ g acetylcholine increased the amplitude and frequency of the Berger waves The effect resembled that obtained during an awakening reaction In the acoustic region there was not only increased spontaneous activity but also an increased after-discharge following acoustic stimuli Larger doses of acetylcholine had a depressant effect The observations have been confirmed by Moruzzi (1939) for the area of mastication in rabbits In addition he found that acetylcholine injections decreased the threshold for cortical stimulation, comparable to the "facilitation" obtained on stimulation of a neighbouring area Miller, Stavraký and Woonton (1940) applied acetylcholine with bits of blotting paper to areas of the motor cortex of cats and rabbits, the result, which resembled a very weak eserine action, was a reduction of the amplitude of the slow waves and it was attributed to asynchronous firing of a large number of neurones On the previously eserinised cortex acetylcholine caused within 2 minutes large and rapid rhythmical waves composed of diphasic spikes, resembling strychnine spikes, with smaller components superimposed The spikes were associated with motor effects on the contralateral side (vibrissae movements, increased tremors and in rabbits movements of mastication) Using the same method, Chatfield and Dempsey (1941) observed no change in electrical cortical activity with acetylcholine alone, but when acetylcholine was given after prostigmine, which itself caused bursts, the individual potentials between the prostigmine bursts became spikes, and new spikes appeared This was not seen in all cortical areas Later fast low potentials appeared Potentials resulting from single shock stimulation of afferent nerves were either not affected or slightly reduced Such stimulation no longer produced the normal sharp localisation of sensory potentials Potentials appeared also in other cortical areas although not, as with strychnine, on the contralateral side When the cortex was isolated from the thalamus, only the fast voltage waves remained, therefore these were thought to represent intrinsic cortical activity, the other components representing activity in thalamocortical circuits

In order to obtain in cats, without eserine or prostigmine, pronounced changes in electrical cortical activity, acetylcholine has to be applied to the cortex in very strong concentrations (2.5 to 10 per cent), acetyl- $\beta$ -methylcholine and carbaminoylcholine are also active, the latter in weaker concentrations (0.08 to

0.3 per cent) The effects, which are localised and persist as long as the drugs are kept in contact with the cortex, resemble the electrical changes recorded from the human cortex during convulsive seizures (Brenner and Merritt, 1942) When large doses of acetylcholine are injected intracisternally into cats or man generalised convulsions occur (Fiamberti, 1937, Brenner and Merritt, 1942) Similar effects produced by intravenous injections of very large doses are, however, not necessarily due to central actions of acetylcholine (Rossi, 1939, Harris and Pacella, 1943, Cohen, Thale and Tissenbaum, 1944)

Williams (1941) found that carbaminoylecholine given subcutaneously to epileptics increased the *petit mal* activity in the electroencephalogram, and that acetylcholine given intravenously sometimes caused epileptic outbursts and electrical changes in the electroencephalogram characteristic of *petit mal* activity

**Sleep** According to Dikshit (1935) acetylcholine (0.1 to 0.5  $\mu$ g) injected into the lateral ventricle of unanesthetised cats, produces a condition resembling sleep lasting for two to three hours Silver and Morton (1936), in similar experiments, observed only drowsiness in their cats In unanesthetised monkeys, the intraventricular injection of a few milligrams of acetylcholine had no effect of this kind (Light and Bysshe, 1936) whereas in man it led to sleep in one, and to drowsiness in two, out of eight patients (Henderson and Wilson, 1936)

**Respiration** An intravenous injection of acetylcholine stimulates, and in larger doses depresses, respiration Whereas the depression is central in origin, the stimulation, when produced in this way, is mainly due to an action of acetylcholine on the chemo-receptors and not, as assumed by Villaret, Besancon and Cachera (1934), to an effect of secreted adrenaline (Schweitzer and Wright, 1938) In atropinised and eserinised cats with chemo-receptors excluded, the effect of intravenous injections of large doses of acetylcholine is no longer mainly depression of respiration, but strong stimulation preceded by inhibition (Schweitzer and Wright, 1938) In dogs with denervated chemo-receptors Heymans, Boukaert and Farber (1935) observed hyperpnoea, sometimes preceded by inhibition of respiration, after arterial injection of acetylcholine Much larger doses were necessary than for stimulation of the chemo-receptors Recently hyperactivity of the respiratory centre after arterial injections of acetylcholine was also observed in dogs by Gesell, Hansen and Worzniak (1942) and by Gesell and Hansen (1943) Subthreshold doses became effective after eserine The response to acetylcholine was essentially a normal hyperpnoea, it was modified by incoming impulses Thus acetylcholine augmented the responses to sinus nerve stimulation or to lung inflation A slow arterial infusion into a dog with denervated carotid bodies produced reinforcement of thoracic respiration, temporary irregularity of "torsal" respiratory movements, associated with subnormal pulmonary ventilation, and initiation or strengthening of the facial respiratory contractions (Gesell and Hansen, 1943)

Dikshit (1934a, b) using intraventricular injection of acetylcholine (0.1-1  $\mu$ g) into unanesthetised cats, usually found temporary arrest, but in some cases acceleration, of respiration Silver and Morton (1936) observed irregularities of respiration under similar conditions in a few cats Miller (1943) found in



creased rate of respiration on local application of acetylcholine (1 in 50 millions) to the floor of the fourth ventricle in decerebrate cats Shuh, Wang and Lim (1936) as well as Gesell, Hansen and Worzniak (1943) observed in anaesthetised dogs hyperpnoea on local application of acetylcholine to the floor of the fourth ventricle, in unanaesthetised dogs the intracisternal injection of 5 mgm carbaminoylcholine stimulated respiration but acetylcholine was ineffective even in much larger doses (Weinberg, 1937) In unanaesthetised monkeys (Light and Bysshe, 1933) and in man (Henderson and Wilson, 1936) the intraventricular injection of a few milligrams of acetylcholine had no appreciable effect on respiration

*Vomiting, coughing, sweating, defaecation and micturition* In man (Henderson and Wilson, 1936) the intraventricular injection of 2.5 mgm of acetylcholine produced nausea, retching, sweating and often vomiting, the centres for lacrimation and salivation were scarcely affected Apart from a slight fall in rectal temperature, due probably to sweating, the temperature was not affected In one instance there was coughing and a feeling of suffocation Despite the regular occurrence of peristalsis, defaecation and micturition took place once only The central origin of sweating was particularly striking in a patient with interrupted sympathetic innervation to the left arm and face, where sweating was absent In some instances the injection appeared only to sensitize the sweat centre, excessive sweating being produced by the stimulus of muscular effort In unanaesthetised cats signs of nausea were observed by Silver and Morton (1936) after intraventricular injections of acetylcholine (20  $\mu$ g) Local application of acetylcholine (1 in 50 millions) to the trigonum hypoglossi beneath the floor of the fourth ventricle caused movements of the tip of the tongue and repeated deglutition (Miller, 1943)

*Pupillary reactions* were not produced by the intraventricular injection of acetylcholine in man (Henderson and Wilson, 1936)

*Bronchi* In curarised dogs Houssay and Orias (1934) found no central vagal effects on the bronchi after arterial injections of acetylcholine

*Heart* Dikshit (1934b) observed that intraventricular injections of 1  $\mu$ g acetylcholine or less into cats under chloralose produced cardiac irregularities similar to those obtained on central vagus stimulation The centre affected must be above the Sherrington level, probably the hypothalamus, because denervation below the Sherrington level abolished the effect Silver and Morton (1936) occasionally observed similar cardiac irregularities According to Gremels (1938) a slow infusion of acetylcholine into the carotid artery of cats increased the excitability of the cardio-inhibitor centre, but the chemo-receptors were not excluded in these experiments In dogs with eliminated chemo-receptors the arterial injection of a large dose of acetylcholine caused bradycardia (Heymans, Boukaert, Farber and Hsu, 1935) The increase in heart rate observed by Weinberg (1937) in large unanaesthetised dogs after intracisternal injection of 90 mgm of acetylcholine may well have been due to an action on the suprarenal medulla after absorption of the acetylcholine In unanaesthetised monkeys the intraventricular injection of a few milligrams of acetylcholine did not change the heart rate (Light and Bysshe, 1933), whereas in man it produced

sometimes slowing and in one instance acceleration (Henderson and Wilson, 1936)

**Blood pressure** Heller and Kusonoki (1933) saw no changes in the blood pressure of cats after intracisternal injection of 5 to 10  $\mu$ g acetylcholine. Dikshat (1934b) in his experiments with intraventricular injections into anaesthetised cats, observed irregular changes and Silver and Morton (1936) observed a small rise followed by a sharp fall, the acetylcholine was injected into the hypothalamic region or into the lateral ventricle. Applied by iontophoresis to the floor of the fourth ventricle acetylcholine caused a rise in arterial blood pressure. This procedure was used as a means of mapping out the pressor area in dogs (Shuh, Wang and Lim, 1936). The pressor effect observed by Weinberg (1937) on intracisternal injection of 90 mgm acetylcholine into large unanaesthetised dogs may have been due to the peripheral actions of absorbed acetylcholine, 5 mgm. carbaminoylcholine, similarly injected, caused intense vasoconstriction but only a slight rise in blood pressure. Haas (1939) denies any central action of acetylcholine on autonomic functions (blood pressure, blood sugar and temperature) because he found it less effective on intracisternal than on intravenous injection. His own experiments were negative but they do not invalidate, as he claims, the results of other workers who used small doses of acetylcholine. The intraventricular injection of a few milligrams of acetylcholine into unanaesthetised monkeys produced a long lasting but moderate fall in blood pressure (Light and Bysshe, 1933) whereas in man it had no effect or caused a slight rise in pressure, but there was some flushing of the skin (Henderson and Wilson, 1936).

**Spinal cord** *Discharge of motor impulses to skeletal muscles* by acetylcholine was suggested by some observations of Feldberg and Minz (1932). In atropinised decapitated cats large doses caused muscular twitching, mainly of central origin, an apparently similar effect was observed by Lefebvre and Minz (1936) when applying acetylcholine on the isolated spinal cord of frogs. Striking evidence for this action was obtained by Bülbring and Burn (1941) in experiments on dogs with separate circulation to the hind limbs and to the spinal cord, the acetylcholine being injected into the latter. Contractions of the limb muscles occurred with 0.06 to 1 mgm acetylcholine and, when eserine was given previously, with 10 and even 1  $\mu$ g. In decerebrate cats Calma and Wright (1944) observed widespread contractions after arterial injections of acetylcholine. In man the intrathecal injection of even 500 mgm acetylcholine was ineffective, but a smaller amount injected with a dose of prostigmine, which by itself would only depress the spinal cord slightly, caused a profound depression (Kremer, 1941).

**Reflexes in somatic nerves** are affected in different ways. Moreover the same reflex may apparently be affected differently in different species and under different experimental conditions, such as anaesthesia, mode of administration, dosage, pre-treatment with eserine, etc. Depression is usually obtained more easily than augmentation. In cats under chloralose the intravenous injection of acetylcholine depresses the *knee jerk* and abolishes strychnine convulsions. The depressant effect on the knee jerk was shown to be mainly one on the spinal cord and to be increased after eserine (Schwetszer and Wright, 1937). A slight

transient increase in muscular tone as well as an initial augmentation of the knee jerk was observed but was attributed to indirect circulatory effects. Recently, however, Calma and Wright found a strong increase in tone of the quadriceps muscle in decerebrate cats owing to a central action of the acetylcholine injected arterially. Carbaminoylcholine caused stronger inhibition of the knee jerk than acetylcholine but acetyl- $\beta$ -methylcholine had no effect at all. Bülbring and Burn (1941) in their experiments on dogs with separated cord circulation occasionally observed an increase of the knee jerk with 20  $\mu$ g acetylcholine, but with 2 mgm it was temporarily depressed and then augmented. After eserine or prostigmine acetylcholine was mainly depressant. When nicotine had abolished the knee jerk large doses of acetylcholine restored it temporarily. The stable choline esters, acetyl- $\beta$ -methylcholine and carbaminoylcholine, caused initial inhibition followed by augmentation. The *flexor reflex* was found to be depressed in cats by intravenously injected acetylcholine (McKarl, Obrador and Wilson, 1939) but slightly augmented in dogs when acetylcholine was injected into the separately perfused cord circulation (Bülbring and Burn, 1941). In the latter experiments depression of the reflex was interrupted by augmentation when acetylcholine was injected after eserine. Unlike the knee jerk, the flexor reflex, however, was never abolished by acetylcholine. The stable choline esters caused augmentation followed by progressive depression.

Bonvallet and Minz (1938b) found that acetylcholine, given intravenously, increased the excitability of the linguo-maxillary reflex. According to these authors (1937a, b, 1938) acetylcholine depresses reflex excitability in spinal animals but increases it in midbrain animals (dogs, cats, rats). The acetylcholine was injected intravenously, or even intraperitoneally, without any precautions to avoid peripheral actions. But similar results were obtained in frogs on application of acetylcholine to the central nervous system applied to the spinal cord it decreased reflex excitability (Lefebvre and Minz, 1936), applied to the midbrain it increased reflex excitability (Bonvallet and Minz, 1938).

Torda (1940) obtained depression of the crossed extensor reflex in spinal toads when they were perfused with strong concentrations of acetylcholine, its peripheral effects being excluded. The depression was counteracted by central stimulation of the intra-abdominal branches of the homolateral sciatic nerve. Reflexes in cats behaved in much the same way (Martini and Torda, 1939) and Bülbring and Burn (1941) obtained similar results in their experiments on dogs with separate cord circulation. After acetylcholine the normal inhibition of the knee jerk, on stimulation of the homolateral posterior tibial nerve, was followed by a large exaggerated rebound.

*Sympathetic spinal centres* In dogs with transverse cord section the intravenous injection of acetylcholine or of carbaminoyl- $\beta$ -methylcholine caused constriction of the separately perfused spleen, which was connected with the dog by its nerves only (Farber and Heymans, 1936). The possibility of an action in the sympathetic ganglia was not excluded.

2 CENTRAL ACTIONS OF ESERINE AND OF PROSTIGMINE Eserine, like acetylcholine, has both stimulant and depressant central effects. If these effects could

be proved to be wholly or mainly due to inhibition of cholinesterase activity they would furnish perhaps the strongest evidence so far available for the acetylcholine theory of central nervous activity. They would prove that acetylcholine is continuously released, that it is normally, either wholly or to a great extent, inactivated by cholinesterase and that the amounts released, when allowed to accumulate, profoundly alter central nervous activity. It could then be assumed that the acetylcholine normally released exerts such functions as are manifest in a more pronounced degree after eserine.

With regard to the *peripheral* actions of eserine it is generally accepted that they are the result of inhibition of cholinesterase. On the other hand if eserine competes with acetylcholine for the same receptor groups of the cholinesterase, it might be expected to compete with acetylcholine in a similar way for the respective functional receptors in gland cells or muscle fibres. Thus the possibility exists of acetylcholine-like actions of eserine independent of the inhibition of cholinesterase. In fact the stimulating action which eserine exerts on some organs when given in extremely large concentrations may be explained in this way. The following facts, however, suggest that the usual peripheral effects of eserine result from inhibition of cholinesterase.

(1) The stimulating action of eserine on smooth muscle is characterised by its long latency, its slow development and its gradual disappearance when the eserine is washed out. These peculiarities point to an indirect mechanism of action.

(2) The smooth muscles of the intestine and of the uterus are both sensitive to acetylcholine but only those of the former to eserine.<sup>1</sup> As a continuous release of acetylcholine and its replacement by synthesis occur in the intestinal wall, probably owing to the activity of the nerve cells of Auerbach's plexus between the muscle layers (references see Feldberg and Solandt 1942) inhibition of cholinesterase must lead to muscular contractions. There is no such nerve plexus and no evidence for a continuous release of acetylcholine in the uterus and therefore eserine if acting solely by inhibition of cholinesterase should not stimulate the acetylcholine sensitive muscle fibres.

(3) Eserine constricts the pupil but not when the postganglionic nerve fibres from the ciliary ganglion have degenerated after its removal although the pupil still contracts to pilocarpine in this condition (Anderson 1905). As first suggested by Gaddum (1936) acetylcholine is released in small amounts from the endings of the ciliary nerve in close proximity to the muscle fibres and accumulates after eserine but after nerve degeneration acetylcholine is no longer released and practically disappears from the iris (Engelhart 1931) which remains however sensitive to the direct action of parasympathomimetic drugs.

(4) Changes in the molecular structure of eserine which lead to disappearance of the inhibitory action on cholinesterase also remove the typical miotic properties on the normally innervated pupil (Stedman 1926).

The assumption that eserine has no direct stimulating peripheral effects leads to new conceptions concerning the function of autonomic ganglia and even of nerve fibres. Eserine slows the heart, even after cutting the vagi, as already shown by Fraser in 1867. The action might result from random impulses travelling down the cut nerve fibres but is more probably due to a continuous release.

<sup>1</sup> In very large concentrations eserine also stimulates the uterus muscle (White and Stedman 1931). The following arguments do not apply to the effects of such concentrations: they may be as mentioned above manifestations of a direct action of eserine.

of acetylcholine by impulses originating in the ganglion cells in the heart. According to this view these cells are weakly automatic comparable to the cells of the plexus of Auerbach. These cells retain the ability to release acetylcholine after degeneration of the pre-ganglionic vagus (Goffart and Bacq, 1939). Of particular interest for the problem of synapses is the action of eserine on motor endplates. It causes fibrillations and fibrillation of skeletal muscles even after severance of the nerves, but the effect disappears two to three days after nerve section (and Kato 1914) i.e., at a time when nerve fibres have lost their ability to release acetylcholine (Feldberg, 1943), but the motor endplates have become more sensitive to acetylcholine. The fascicular eserine twitches may be accounted for by the release of acetylcholine by random impulses passing the cut nerve (Bacq and Brown, 1937), the fibrillations cannot be explained in this way. They may be due to a continuous release and resynthesis of small amounts of acetylcholine from the endings of the "resting" nerve, so slight twitches have no effect in the absence of eserine (Feldberg, 1945). According to this view the motor endplates are always insensitive to the direct action of eserine.

There is thus strong evidence that the peripheral effects of eserine are due to its action on acetylcholine effects and that apart from its inhibitory action on cholinesterase, eserine is a pharmacologically inert substance. We have dealt in such detail with the peripheral effects because the assumption of a different mechanism for the central effects of eserine would be unnecessary if the conditions for an indirect mechanism of action prevail also in the central nervous system. This is the case. The central actions of eserine resemble those of acetylcholine sufficiently to be explained by the accumulation of the latter. Acetylcholine is present in the central nervous system and apparently is continuously released and replaced by synthesis, the tissue contains cholinesterase, therefore acetylcholine is bound to accumulate and exert its effects in the presence of eserine.

There are, however, certain difficulties. Under apparently similar conditions some inhibitors of cholinesterase may be excitatory, others depressant, even the same substance in the hands of different workers may have opposite actions. A review of the old as well as of the new literature reveals this strange pharmacological irregularity which has led to as yet unresolved controversies. Schwartz, Stedman and Wright (1939) suggested that the inhibitors of cholinesterase which are tertiary bases, such as eserine, give rise in the body to a lipoid-soluble base which penetrates the cells and acts as a convulsant, whereas the quaternary bases such as prostigmine, give rise to a lipoid-insoluble substance which remains outside the cells and acts as a depressant. This explanation could account for their results and those of Kremer (1941) but not for those of most other authors. The same inhibitor of cholinesterase was found to have opposite actions at different levels in the central nervous system (Bonvallet and Minz, 1938, McKelvey, Obrador and Wilson, 1939), in the spinal cord the response varied with the reflex tested but there was no qualitative difference between eserine and prostigmine (Merlis and Lawson, 1939; McKelvey, Obrador and Wilson, 1939, Bülbirner

and Burn, 1941) The anesthetic used and the mode of application seemed to influence the response Most authors found a similarity of action between acetylcholine and eserine or prostigmine and attributed it to the cholinesterase inhibiting property of these substances The following points strengthen this view

(1) There is a relationship between the inhibition of cholinesterase activity of a substance *in vitro* and its central effects (Schweitzer and Wright 1939) in spite of the fact that the substances examined are decomposed by cholinesterase at widely different rates (Easson and Stedman 1936) so that certain discrepancies are to be expected Particularly striking were the results obtained on removal of the urethane grouping. Eseroline and eseroline methiodide derived from eserine and eserine methiodide respectively had a feeble inhibiting action on cholinesterase *in vitro* and exerted only weak central actions Removal of the urethane grouping from prostigmine considerably weakened but did not abolish its central inhibitory action on the knee jerk Schweitzer and Wright conclude that central inhibition may be partly independent of inhibition of cholinesterase, whereas central excitation is wholly due to it Mention may be made in this connection of the fact that eserine in large doses apparently has a direct paralyzing action on the cells of the sympathetic ganglion (Feldberg and Vartiainen, 1934)

(2) As in the peripheral effects the time course of the response to eserine indicates an indirect mechanism of action. There is a definite latency which varies according to the mode of administration and the doses used but which is so far as comparisons have been made longer than that seen with acetylcholine under similar conditions For instance the onset of the response to intraventricular injections of a few gamma eserine is delayed for 12 minutes that to acetylcholine for 30 seconds (Henderson and Wilson 1936) With very strong concentrations of eserine (1 to 10 per cent) applied locally to the motor cortex there is a time lag of 6 to 7 seconds before motor activity sets in (Miller, Stavsky and Wootton 1940) The effect on the knee jerk in rats started three minutes or more after the intravenous injection of a small dose of eserine and reached its maximum slowly (Schweitzer and Wright 1937 1938b) with larger doses the latency progressively shortened Bülbring and Burn (1941) published tracings of the effects on reflexes of drugs injected into the spinal circulation acetylcholine nicotine and adrenaline acted almost immediately i.e. within the first ten seconds and the effect quickly reached a maximum With eserine or prostigmine there was a latency of 20 to 30 seconds and the effect increased gradually during the next minute or two Gessell and Hansen (1943) point out that the eserine hyperpnœa develops slowly and disappears slowly, reflecting a gradual accumulation and disappearance of acetylcholine The effects of subcutaneously injected eserine and prostigmine on the *petit mal* activity in epileptics occurred after a latency of 20 minutes or longer (Williams and Russell, 1941) There was an even longer latency before the effects on the spinal cord developed in man after intrathecal injections of eserine or prostigmine (Kremer, 1941)

*Literature before 1934* Most of the early workers, using more or less impure preparations, observed effects which were attributed to central depressant and excitatory effects Fraser (1867) who discovered the pharmacological potency of physostigmine, attributed the respiratory failure produced by the active principle of the calabar bean to a central action of the drug It also produced a slight evanescent rise in body temperature Fraser described many experiments which show that eserine causes paralysis of the spinal cord in frogs, rabbits and dogs leading to complete loss of reflex function No effects were observed when the drug was applied directly to the cerebral cortex of frogs, birds and mammals, when it was applied to the spinal cord peculiar twitches occurred in the limbs

followed by paralysis Strychnine convulsions were abolished or prevented He believed "that no other drug so directly diminished reflex action" as physostigmine and recommended extracts of the calabar bean against epileptic fits and tetanus Its use was also recommended by other authors against these and other conditions of increased reflex excitability, strychnine poisoning and chorea, for references, see Harnack and Witkowski (1876) These authors, on the other hand, showed that a subcutaneous injection of physostigmine into an epileptic idiot led to numerous fits and psychic disturbances and that fits closely resembling those of epilepsy may be produced in specially operated guinea pigs Brown-Séguard (1860) had shown that a convulsive affection resembling epilepsy can be produced by certain lesions in the spinal cord, subcutaneous injection of physostigmine into such animals was followed by an enormous number of fits during the next hours

According to Harnack and Witkowski pure physostigmine had a central depressant effect only, the preceding stage of excitation observed by numerous workers was attributed to impurities Harnack and Witkowski themselves however, observed this initial excitation with pure physostigmine Heubner (1905) found that physostigmine usually caused depression in frogs, but sometimes convulsions which with one preparation were produced as regularly as with strychnine In mammals excitation preceded paralysis, epileptic fits were produced in dogs and could be suppressed by chloroform Rothberger (1901) concluded that excitation was probably due to cortical stimulation and that physostigmine, in suitable doses, stimulated the respiratory centre The depression observed in frogs affected first voluntary movements, then reflex movements and finally respiration According to Harnack and Witkowski motor and sensory regions were affected in mammals, according to Roerber (1868) there was complete paralysis of the nerve cells for pain whereas those for touch and muscle sense remained unaffected by physostigmine Strong central excitation was obtained with physostigmine by Langley and Kato (1914) In cats and rabbits twitching of the eyelids and nystagmus occurred occasionally, synchronous on both sides and, therefore, undoubtedly central in origin Similar synchronous movements and other muscular contractions were observed in birds The brief rise in arterial blood pressure and the secretion of sweat from the cat's pad observed after physostigmine were thought to be mainly central in origin They concluded that small doses of physostigmine set up new nerve impulses but they left open the question whether these were in part or wholly due to increased irritability of central synapses Stewart and Rogoff (1921) described a centrally produced secretion of adrenaline by eserine which, however may have been caused reflexly and not by a direct action of the eserine on the central nervous system

*Literature after 1934 Cortex* Miller (1937), using anaesthetised cats, placed bits of blotting paper soaked in 1 or 10 per cent of eserine solution on the area of the motor cortex for the fore and hind leg The muscles of the contralateral leg contracted, the leg became rigid and during the following minutes tremors and a gradually increasing clonus set in Excision of the eserinated motor cortex promptly softened the affected leg The response to eserine was stronger

than that to faradisation of the cortex, which sometimes consisted mainly of flexion while that to eserine consisted mainly of extension. Tremors and clonus were specific for eserine. Removal of the blotting paper caused the symptoms to subside after several minutes, afterwards the threshold for cortical faradisation was lowered and flexion or clonus sometimes continued as after discharges. Later Miller, Stavraky and Woonton (1940) found that local application of eserine to the motor cortex of cats and rabbits caused within a minute or two a progressive reduction in amplitude of the slow and large fast waves in the electroencephalogram: the small fast waves and the dot waves became more apparent. These changes being associated with the described motor phenomena represented cortical stimulation. The reduction in amplitude was attributed to asynchronous firing of large numbers of neurons, owing to stimulation of multitudes of synapses in widely different degrees. Sjöstrand (1937) had previously stated that eserine applied to the cortex together with strychnine was more active than strychnine alone in evoking large diphasic waves from the cerebral cortex. Chatfield and Dempsey (1941) applied 1 per cent prostigmine to the cerebral cortex of cats and observed depression of electrical activity, sometimes localised to the area of application, sometimes spreading to remote areas, later, spontaneous bursts appeared. Williams and Russell (1941) injected small subcutaneous doses of eserine and prostigmine into epileptics, prostigmine usually increased, whereas eserine decreased, the *petit mal* activity recorded in the electroencephalogram. As *petit mal* activity may well be the result of depression of nervous activity, the results are not necessarily at variance with the theory of Schweitzer et al. concerning quaternary and tertiary anticholinesterases. There was no effect on the encephalogram of normal persons. The intrathecal injection of prostigmine and eserine in man sometimes caused headache and in one case disturbances of speech (Kremer, Pearson and Wright, 1937, Kremer, 1941). Unlike prostigmine, eserine produced striking sensory changes including facilitation of sensory transmission (Kremer, 1941).

The effects observed in the isolated cat's head perfused with diluted blood may be due to cortical stimulation: eserine added to the blood caused hyperexcitability of the existing reflexes or their reappearance if they had disappeared, spontaneous blinking at the rate of about 80 per minute and jerking movements of the head sometimes occurred, the effects disappeared after section of the nerves, at later stages of eserine poisoning, depression set in (Chute, Feldberg and Smyth, 1940). Increased reflex excitability of the head was also obtained after injection of eserine into the carotid artery of cats (McKail, Obrador and Wilson, 1941), at the same time the motor response of limb and face muscles to electrical stimulation of the cortex was depressed. There was some suggestion that eserine occasionally potentiated the cortical response to central vagus stimulation.

**Cerebellum** In one decerebrate cat Miller (1937) placed tablets of eserine near the centre of the anterior lobe of the cerebellum "almost at once the right foreleg was thrust forward, the left likewise but more slowly. The hindlegs relaxed promptly then stiffened, later relaxed and stiffened alternately."

**Brain stem, medulla** In man the intraventricular injection



dose which was ineffective intravenously, produced repeated vomiting, slight sweating and slowing of the pulse (Henderson and Wilson, 1937) Kremer (1941) observed vomiting, sweating, pallor and drowsiness after intrathecal injection of both eserine and prostigmine, emptying of the bladder was abolished

*Respiratory centre* Both prostigmine and eserine, given intravenously to cats under chloralose anesthesia, depressed respiration, the prostigmine depression was sometimes preceded and followed by stimulation Cutting the vagi and eliminating the chemo-receptors did not materially alter the response But in decerebrate cats the more pronounced stimulating effect of prostigmine on respiration was mainly a response of the chemo-receptors Depression was observed with several other quaternary anticholinesterases (Schweitzer and Wright, 1938, 1939) In anesthetised dogs with eliminated chemo-receptors eserine, injected into a vertebral artery, caused a slowly developing hyperpnoea, sometimes preceded by diminished ventilation, return to normal respiration required hours (Gesell and Hansen, 1943) The effect resembled that produced by a slow arterial infusion of acetylcholine The authors emphasize the difference between inhibition and paralysis of the centre when eserine had caused arrest of respiration by inhibition, stimulation of the sinus nerve produced a more powerful response than before eserine, but after excessive eserine poisoning the centre became paralysed and no longer responded to afferent impulses The stimulating action of eserine and prostigmine on "the crossed phrenic phenomenon" is probably a central effect of these drugs, but a possible action on the chemo-receptors was not excluded (Seligman and Davis, 1941)

Dikshit (1934) found no change in the respiratory response to central vague stimulation in cats, when eserine was given intravenously or intraventricularly, but Gesell, Hansen and Worzniak (1942) found in dogs that subthreshold doses potentiated the response to stimulation of the sinus or of the superior laryngeal nerve

*Spinal cord Spontaneous discharge* Lefebvre and Minz (1936), by applying eserine locally to the isolated spinal cord of frogs, observed a change in reflex excitability suddenly followed by a discharge of motor impulses In both anesthetised and decerebrate cats after atropine the intravenous injection of large doses of eserine caused convulsions and a marked increase in muscular tone The effects were abolished after nerve section and were due to an action of the spinal cord as they were seen equally well after its division in the midthoracic region Smaller doses caused increased reflex excitability Other inhibitors of cholinesterase were examined, tertiary amines acted like eserine but quaternary bases, like prostigmine, depressed the cord and were able to abolish temporarily eserine and strychnine convulsions (Schweitzer and Wright, 1937, Schweitzer, Stedman and Wright, 1939, Calma and Wright, 1944) In dogs Merlis and Lawson (1939) observed a spontaneous discharge confined to myotomes innervated from the cord segments perfused with eserine Increase of muscle tone was obtained after intravenous injection of a large dose of eserine Bülbring and Burn (1941) observed a spontaneous discharge in dogs after injecting eserine

or prostigmine into the separate cord circulation. Nicotine, which had no action of this kind when given alone, caused a spontaneous discharge after subthreshold doses of these drugs.

*Reflexes. Knee jerk.* Schweitzer and Wright (1937, 1938c) and Schweitzer, Stedman and Wright (1939), using cats anaesthetised with chloralose, found that the intravenous injection of eserine and other tertiary anticholinesterases augmented the knee jerk whereas prostigmine and other quaternary anticholinesterases depressed it. The increased excitability to eserine was often preceded and sometimes followed by a phase of diminished reflex activity. When the knee jerk had been abolished by an overdose of the anesthetic or by ergotoxine or prostigmine it was restored and even enhanced by eserine. In most of their experiments atropine was given intravenously to avoid the circulatory effects of eserine. The same difference in the action of eserine and prostigmine was observed in man after intrathecal injection by Kremer (1941). In a case of spinal block where the reflex was absent it could be evoked after eserine. Merlis and Lawson (1939) worked on dogs usually with the cord transected, they perfused the subarachnoid space or applied the eserine locally to the cord and found that the knee jerk was usually depressed. After intravenous injection of eserine the reflex was augmented in over 40 per cent of experiments when chloralose and in 10 per cent when barbital was used as the anesthetic. Bülbring and Burn (1941) observed depression and abolition of the reflex after eserine or prostigmine had been injected into the separately perfused cord circulation of dogs, in spite of the fact that the drugs caused spontaneous discharges. Eserine, however, exaggerated the rebound which sometimes followed the inhibition of the knee jerk caused by homolateral stimulation of the posterior tibial nerve. The effects of nerve stimulation resembled those of injections of acetylcholine both before and after eserine. *Flexor reflex.* In cats, augmentation of this reflex occurred after intravenous injection of eserine (McKail, Obrador and Wilson, 1941). In dogs the reflex was examined by Merlis and Lawson (1939) and by Bülbring and Burn (1941). After intraspinal injection, eserine, even in large doses, always augmented the reflex. After intravenous injection, Merlis and Lawson observed depression in 5 per cent of cases. Prostigmine had a much weaker stimulating action which was only obtained in the presence of adrenaline in the cord circulation. In man (Kremer, 1941) intrathecal injection of eserine increased, and that of prostigmine abolished flexor reflexes. *The crossed extensor reflex* was increased in dogs after eserine (Merlis and Lawson, 1939). Torda perfused spinal toads from the aorta with concentrated solutions of prostigmine, but eliminated peripheral effects. As with acetylcholine the crossed extensor reflex was depressed and was no longer inhibited by homolateral stimulation of the sciatic nerve, which now evoked a large response. The results resemble those obtained by Bülbring and Burn on the knee jerk.

*Other reflexes and spinal activity.* In frogs eserine diminished the height of the reflex twitch of the semitendinosus provoked by homolateral stimulation of the sciatic nerve, but augmented the after discharge in the electromyogram (Bonnet and Bremer, 1937a). McKail, Obrador and Wilson (1939) found that eserine

usually depressed the response to stimulation of the pyramidal tract, like that to stimulation of the cortex, but in one instance increased the pyramidal tract response. In man (Kremer, Pearson and Wright, 1937, Kremer, 1941) muscle tone and reflexes were depressed by prostigmine injected intrathecally and the voluntary muscle movements were impaired, but in patients with hemiplegia these improved on the affected side, probably due to abolition of spasticity. The impairment of micturition also might have been a spinal effect. In a case of spinal block, prostigmine injected below the level of the block abolished muscle tone, spasm and reflexes. The effects of eserine however were mainly excitatory. After an initial depression of the spinal reflexes they were augmented with increased power in the legs, although no muscle spasm could be seen or felt. In the case of spinal block which displayed complete flaccid paralysis of the legs and loss of reflexes, the latter were re-established after eserine.

3 THE EFFECTS OF ATROPINE ON THE CENTRAL ACTIONS OF ACETYLCHOLINE, AND ESERINE PROSTIGMINE. Most authors have found that atropine antagonises the stimulating as well as the depressant central actions of acetylcholine, eserine and prostigmine, although there is a number of contrary statements. For instance, Dikshit (1934) injected atropine intravenously or intraventricularly without observing any effect on the inhibitory action of acetylcholine applied to the respiratory centre, similarly Shuh, Wang and Lum (1936) found that atropine, injected intravenously, was without effect on the stimulating action of intraventricular injections of acetylcholine on blood pressure and respiration. Farber and Heymans (1936) could not abolish by means of atropine the spleen contraction of central origin produced by acetylcholine. Schweitzer and Wright, Schweitzer, Stedman and Wright and Calma and Wright performed most of their experiments with anticholinesterases on atropinised cats because they had found that atropine did not materially alter the result. Merlis and Lawson (1939) found that neither the depression of the knee jerk nor the enhancement of the flexor reflex caused by eserine was affected by atropine, Kremer (1941) found atropine ineffective on the responses in man to intrathecal injections of eserine and prostigmine. Similarly Brenner and Merritt (1942) found that intravenous injections of atropine had no influence on the responses produced by acetylcholine applied in strong concentrations to the cerebral cortex or injected intracisternally.

The following observations, however, provide evidence for an antagonistic effect of atropine. Langley and Kato as early as 1915 had stated that the irregular eserine contractions of the limbs, which were central in origin, did not occur after atropine. According to Schweitzer and Wright (1937) atropine usually decreased or abolished the paralysing effect of acetylcholine on the knee jerk, according to Calma and Wright (1944) it abolished the augmentor effect of acetylcholine on the muscle tone in decerebrate cats. Atropine abolished the changes observed by Bonvallet and Minz (1937, 1938) in reflex excitability produced by acetylcholine in spinal and mid brain animals as well as the increase in excitability of the linguo-maxillary reflex produced by acetylcholine or eserine in dogs and cats (1938b). Bülbring and Burn (1941) found that atropine

abolished both the discharge of motor impulses from the spinal cord and the changes in reflex excitability produced by acetylcholine eserine or prostigmine. According to Schweitzer and Wright atropine diminished the depressant effect of acetylcholine on the respiratory centre, after atropine only large doses were effective. Gesell and Hansen (1943) found that atropine reversed each of the changes in breathing which had been set up by eserine or acetylcholine. Miller (1943) found that intravenous injection of atropine abolished the effects on deglutition, respiration and tongue muscle produced by local application of acetylcholine in low concentration to the floor of the fourth ventricle. Similarly atropine prevented or abolished the stimulating effects of intraventricular injections of acetylcholine or eserine in man (Henderson and Wilson, 1936). The changes produced by acetylcholine in the response of the cortex to electrical stimulation were abolished by atropine, but the changes produced by eserine in the response to cortical or pyramidal tract stimulation were not wholly antagonised. Once eserine had abolished the electrical response atropine could not restore it (McKail, Obrador and Wilson, 1939). According to Miller, Stavratsky and Woonton (1940) atropine given intravenously or applied to the cortex prevented the changes in the electroencephalogram produced by the local application of acetylcholine or eserine, whereas Chatfield and Dempsey (1941) found that atropine injected intravenously into cats, abolished the spikes, which occurred in the intervals between bursts, but none of the other many changes in cortical activity produced by local application of strong solutions of acetylcholine or prostigmine. The effects of eserine, prostigmine, carbaminoylcholine and acetylcholine on the *petit mal* activity of the cortex in epileptics were abolished by atropine (Williams and Russell, 1941, Williams, 1941).

*Central effects of atropine when given alone* It is well known that atropine has stimulating effects on the medulla and higher centres and only ultimately causes central depression. There is at present no justification for interpreting the stimulating action of atropine as an abolition of central depressant actions of released acetylcholine. There is another well known action of atropine, its sedative effect on the rigidity and tremor of parkinsonism. It is tempting to regard this effect as a central atropine-acetylcholine antagonism, similar to that observed when both drugs are applied artificially to the central nervous system. In fact there are a few recent observations suggesting that atropine may abolish central activity. Schweitzer and Wright (1937), unlike Bülbring and Burn (1941), found depression of the knee jerk on intravenous injection of atropine in doses not exceeding those necessary to abolish parasympathetic nerve effects. Often the depression was pronounced and no recovery took place. Bonvallet and Minz (1938b) saw inhibition of the linguo-maxillary reflex on intravenous injections of atropine into dogs and cats and Gesell and Hansen (1941) observed appreciable diminution of breathing when small doses of atropine were injected into the vertebral artery of dogs with eliminated chemoreceptors and double vagotomy. McKail, Obrador and Wilson (1939) describe a definite effect of atropine on cortical activity: the motor response to electrical cortical stimulation was depressed by  $\text{CO}_2$  and by central vagus stimulation atropine abolished the

CO<sub>2</sub> depression and mildly antagonised that caused by central vagus stimulation. Brenner and Merritt (1942) found that atropine applied to the normal cortex produced changes in electrical activity very similar to those produced by atropine on the eserinated cortex

Despite these few observations the discrepancy remains that whereas atropine usually abolished the central effects of acetylcholine, applied artificially or allowed to accumulate by the action of eserine or prostigmine, it has little effect on the spontaneous and reflex activity of the central nervous system. Such a discrepancy has been observed also for peripheral effects and has been explained (Dale and Gaddum, 1930) on the assumption that the acetylcholine may be released in immediate contact with, or even inside, the cell membrane and that atropine is unable in these circumstances to intervene with its antagonistic action. This problem has been dealt with in detail by Dale (1938). With regard to the central nervous system the discrepancy is in fact not so great as in the peripheral nervous system because the atropine-acetylcholine or eserine antagonism was not obtained by all authors and because some central activity is abolished by atropine.

Atropine is not the only drug which abolishes the peripheral actions of acetylcholine. Scopolamine which is closely related chemically to atropine has a similar effect. If scopolamine had been used as widely as atropine has been in elucidating the mechanism of action of peripheral cholinergic nerves the parallelism between the peripheral and central actions of acetylcholine and nervous activity would have been much closer. Scopolamine, however, has not been examined as an antagonist to the central actions of acetylcholine, eserine or prostigmine.

There are other drugs which ought to be re-examined in the light of the theory of acetylcholine as a central transmitter. The actions of adrenaline have been reviewed recently (Burn, 1945) in this journal from the point of view of the acetylcholine theory and therefore will not be dealt with in this article.

**4 ACETYLCHOLINE CONTENT OF THE TISSUE OF THE CENTRAL NERVOUS SYSTEM**  
Chang and Gaddum (1933) were the first to demonstrate with reliable pharmacological tests the presence of acetylcholine in brain and to give quantitative figures. In the next two years Dikshit (1934), Plattner (1934) and Kwiatkowski (1935) showed the presence of acetylcholine and its uneven distribution in the central nervous system. According to Dikshit, the cerebral cortex contained a little, the cerebellum much less acetylcholine than the basal ganglia, Kwiatkowski found more in the thalamus region than in the cortex. Some of the acetylcholine values given by these early workers are probably incorrect because they used methods which did not extract all the acetylcholine or allowed loss or synthesis to occur during the procedure. This argument applies also to the values given by Corteggiani, Gautrelet, Kaswin and Mentzer (1936) and by Fegler, Kowarzyk and Lelusz-Lachowicz (1938). In order to extract all the acetylcholine without loss or new formation the tissue has to be extracted either with well acidified (HCl) saline and boiled or with trichloroacetic acid. The mincing and grinding must be carried out in these solutions since, if carried out in saline or in eserinated

saline, loss or new formation of acetylcholine respectively may occur. The final neutral extract must be assayed not against pure acetylcholine solutions, as is usually done, but against acetylcholine solutions containing equivalent amounts of tissue extract, the acetylcholine content of which has been destroyed by short treatment with alkali, otherwise the values obtained may be too high, particularly when the acetylcholine content of the tissue is low.

Acetylcholine has been isolated and identified chemically from the optic ganglion of octopus (Bacq, 1935) and later from ox brain which had been minced and incubated in chloroform before extraction to increase the yield of acetylcholine by synthesis (Stedman and Stedman, 1937).

The acetylcholine content of different parts of the central nervous system compared with values obtained from the peripheral nervous system is given in table 1 which summarises roughly the results obtained by the following workers: Chang and Gaddum (1933), Plattner (1934), Barsoum (1935), Bacq (1935), Dikshit (1938), Loewi and Hellauer (1938), Haas (1939), MacIntosh (1939, 1941), Cortelli, Feldman and Gellhorn (1941), Feldberg (1943, 1945), Welsh (1943). MacIntosh who supplied most of the values obtained in dogs and cats summarises his results as follows: no part of the central nervous system is as rich in acetylcholine as some peripheral nerve trunks or sympathetic ganglia. Acetylcholine is found both in grey and white matter of the cerebral hemispheres, but in all regions the grey matter contains more than the white. In the spinal cord the acetylcholine is mainly confined to the grey matter. The afferent fibres in the dorsal columns and in the medulla and, what is more surprising, the pyramids contain little or no acetylcholine. It occurs in fairly high concentration in the basal ganglia and in the midbrain. This might suggest concentration of acetylcholine at central synapses or in relation to cell bodies. On the other hand there is a significant proportion of acetylcholine in parts of the corpus callosum, of the internal capsule and in the superficial layers of the pons, all of which contain few cell bodies, while the cerebellum which is rich in cells contains hardly any acetylcholine. Its presence in many parts of the central nervous system does not necessarily signify the presence there of cholinergic neurons, as MacIntosh points out, and its absence does not definitely exclude their absence, although it makes it more probable. MacIntosh assayed samples of ascending and descending tracts of the frozen spinal cord of horses in order to obtain more detailed knowledge, but the acetylcholine content was too low to give reliable information.

A low store of acetylcholine with a great ability to synthesise it may be a characteristic feature of a tissue which like the central nervous system exhibits continuous activity. When considering the theory of acetylcholine as central transmitter it is necessary to realize that the acetylcholine content of brain apparently decreases with the phylogenetically higher development of the species.

The acetylcholine content of individual brains of the same species varies greatly but not that between the right and left halves of the same brain. On the average large guinea pigs contain a higher concentration of acetylcholine in

their brains than small ones, suggesting an increase with age (Feldberg, 1945). This result is supported by experiments made by Welsh and Hyde (1944). They ground rat's brain in ice cold eserised saline solution for a given time thus bringing an unknown proportion of the tissue acetylcholine into solution. They used brains of new born, young and adult rats and found that the amounts of acetylcholine increased with age, but to speak of this acetylcholine as free acetylcholine of the brain is misleading. Results obtained by the same method suggest that the acetylcholine content of the different parts of brain changes with age. In new born rats it appeared to be lowest in the pallidum and highest in the medulla, in adult rats lowest in the cerebellum and highest in the brain stem. It is unfortunate that the authors made no determinations of the actual acetylcholine content of the tissues. Welsh and Hyde point out that there is a general agreement in the quantitative distribution of acetylcholine in the central nervous system of the growing rat and that of the developing chick as determined by Szepeswol and Caretti (1942). In the early chick embryo the medulla contains much acetylcholine which diminishes with age, in the diencephalon and mesencephalon the acetylcholine increases from a medium amount in the early stages to a high level which is maintained in the adult bird, the cerebrum at first has very little acetylcholine, but this increases and then diminishes again, the cerebellum shows a medium amount of acetylcholine in the early stages which decreases in the older chick. According to Kuo (1939) acetylcholine appears in chick embryos before synapses are found and there is no relation between increase in acetylcholine and development of the reflexes or of the nervous system.

*The form in which acetylcholine is present in the nervous tissue* If acetylcholine were not protected against cholinesterase it could be present in the tissues in traces only, if at all. To explain this protection it might be assumed that acetylcholine is present in a diffusible form inside the cell but spatially separated from the tissue cholinesterase. The cell would form a diffusion-tight compartment and the release of acetylcholine could be visualised as an increase in the permeability of the membrane, injury of the membrane would bring acetylcholine into solution and into contact with cholinesterase so that it would be destroyed at once. This conception, as pointed out by Beznak (1934), is certainly wrong. When nervous tissue is divided mechanically by grinding it in sand, mincing or even homogenising it, the cell debris which can be spun down by centrifugation or seen under the microscope retains acetylcholine. Some acetylcholine is freed in the process and brought into solution, the debris, however, by synthesis replaces its acetylcholine so that on extraction it yields its normal complement. The wrong interpretation of this observation led to the assumption of an inactive "precursor substance" or of two kinds of acetylcholine, "bound" and "free", present in the tissue. As cell debris does not dialyse it is not surprising that the so-called precursor substance was found to be non-dialysable. By boiling a suspension of finely divided tissue or treating it with acid the cell debris is destroyed and its acetylcholine brought into solution, consequently the activity of such extracts increases. This is the explanation of the results obtained by Corteggiani and her co-workers, as pointed out by Trethewie (1938).

Acetylcholine is bound in the tissue or cell granules to some cell constituents,

it is synthesized into this linkage and in this condition is relatively immune to cholinesterase. The cell constituents responsible for the linkage are thought to be the lipins as well as the proteins. Chang and Gaddum (1933) observed that alcoholic extracts were less active when tested for acetylcholine than trichloroacetic acid extracts, but Barsoum (1935) found that the deficit could be recovered when the alcoholic extract was subsequently treated with trichloroacetic acid. An alcoholic extract, first freed from alcohol and then shaken with ether to remove fats, contained a certain amount of insoluble material in suspension. Such extracts when kept overnight lost about half their activity which, however, could be recovered by treating the extract with trichloroacetic acid. The disappearance of activity was apparently due to the formation of an inactive compound. No active substance was formed from lecithin when treated with trichloroacetic acid. Similar results were obtained by Loewi and Hellauer (1938) using tissue of frog's central nervous system. Acid alcohol extracts were found to be only half as active as trichloroacetic acid extracts, nevertheless all the acetylcholine of the tissue had been extracted, but half of it had been converted into a saline insoluble form from which it could be freed and made water soluble by trichloroacetic acid. They found that acetylcholine, which is practically insoluble in ether, became very soluble in ether when it was added to an alcoholic extract of nervous tissue and that the acetylcholine of the tissue could be removed to about 80 per cent from an acid alcoholic extract by the addition of ether. The ether solution obtained in this way showed slight opalescence. Acetylcholine added to acid alcohol without nervous tissue did not become ether soluble (Loewi, Hagen, Kohn and Singer, 1937, Loewi and Hellauer, 1938). From these facts they concluded that acetylcholine is linked in the tissue to the lipoids, this linkage would not explain why acetylcholine cannot be extracted from the nervous tissue by ether without a preceding extraction with acid alcohol, therefore they assumed an additional linkage to another tissue constituent, probably to the proteins.

In whatever linkage the acetylcholine is held we have to assume a kind of dynamic equilibrium in order to explain the findings of an apparently fixed acetylcholine content of the tissue. The tissue is able to store a definite but limited amount of acetylcholine which may be determined by the number of available cell constituents to which the acetylcholine is linked (see also section 8). Nevertheless synthesis is proceeding continuously at a slow rate, the excess of acetylcholine being released and at once destroyed. There is no evidence that "free" acetylcholine exists or can exist in the tissue. Even the tissue acetylcholine may not be completely immune to cholinesterase, but synthesis, as long as it proceeds, compensates any loss that may occur through enzymatic hydrolysis and thus allows the acetylcholine to attain and maintain certain definite levels characteristic for each part of the nervous system, for each species and for the age of the animal. When synthesis, however, is impaired or lost, the acetylcholine disappears from the tissue, as is the case with peripheral nerves in the early stages of degeneration (Feldberg 1943) or with brain tissue kept frozen for several days (Feldberg 1945).

Some of the protection of the tissue acetylcholine against the cholinesterase



is apparently lost when brain tissue is perfused for several minutes with saline solution devoid of eserine, it was found that the acetylcholine content of the guinea-pig's brain decreased under such circumstances, but when eserine was present in the perfusion fluid it remained normal. Similarly the cell granules of brain pulp gradually lose part of their acetylcholine when kept for some time in saline solution without eserine, in presence of eserine, however, no apparent change in their acetylcholine content occurs (Feldberg, 1945). In view of these observations the findings of Cortell, Feldman and Gellhorn (1941) that an intravenous injection of eserine into rabbits increases the acetylcholine content of the brain needs confirmation.

It is possible that the lipins, apart from any rôle they may play in the linkage of acetylcholine, confer upon the tissue acetylcholine its relative immunity to cholinesterase. Such an assumption would explain the fact that cell debris of fresh brain pulp, which on suspension in saline solution retains its acetylcholine store for some time, no longer does so when ether is also present. In presence of ether eserine must be added in order that the particles may retain their acetylcholine or regain it when it has disappeared through the absence of eserine (Feldberg, 1945).

Brain tissue dried *in vacuo* and powdered is unable to build up a store of acetylcholine in its particles when it is suspended in eserinised saline solution, but it acquires this property when suspended in an eserinised saline-ether mixture (Feldberg, 1945).

**5 SYNTHESIS OF ACETYLCHOLINE** Synthesis of acetylcholine by tissues of the central nervous system was discovered independently by Quastel, Tennenbaum and Wheatley (1936) and by Stedman and Stedman (1937). It occurs also in tissue of the peripheral nervous system and is a property not only of the nerve cell but also of the nerve fibre. The only non-nervous tissue in which it has been observed with certainty is the human placenta (Feldberg, 1943, 1945). Mention, however, may be made of the fact that bacteria (*Bacterium acetylcholini*) are able to form acetylcholine (Keil and Kritter, 1935, Keil and Weyrauch, 1937/38, Möller and Ferdinand, 1937/38, Habs, 1937/38, Möller, 1938).

The synthesis of acetylcholine has been studied under the following three conditions

- (1) when brain slices or pulp are incubated in a saline medium,
  - (2) when brain pulp or dried powdered brain is suspended in a mixture of saline and chloroform or saline and ether respectively
- and (3) when homogenised brain or a saline extract of acetone dried and powdered brain is used as the material for synthesis

In all these conditions it is necessary to add eserine or another inhibitor of cholinesterase to the medium in order to prevent the hydrolysis of the acetylcholine formed, although it should be pointed out that the saline extracts from acetone dried brain contain only mere traces of cholinesterase. Under the third condition the enzyme system responsible for the synthesis is in solution and the acetylcholine is synthesized as free acetylcholine. No release of ace-

tylcholine from tissue particles is possible and synthesis therefore can be examined without interference from this factor. Under the first two conditions the enzyme system is still attached to the particulate matter, although not necessarily to intact cells. The acetylcholine is synthesized within the cells or cell granules and before being brought into solution must be released from them. As mentioned above the tissue particles maintain their normal store of acetylcholine, or rather have the tendency to replace it as long as synthesis is allowed to proceed, but they appear to be unable to build up a store of acetylcholine higher than their normal physiological complement. Under these conditions, therefore, synthesis is closely linked with and dependent upon the release of acetylcholine and it is not always easy to distinguish clearly between effects promoting release and formation respectively. When brain slices or pulp are incubated in a saline medium synthesis occurs under conditions where respiration apparently is a dominant factor, on the other hand when brain pulp or dried brain is suspended in a mixture of saline and chloroform or saline and ether respectively, or when dried brain is suspended in saline solution synthesis occurs under conditions where respiration is more or less absent.

(1) *Brain slices or pulp in a saline medium* were used first by Quastel, Tennenbaum and Wheatley; the method has been worked out in more detail by Mann, Tennenbaum and Quastel (1938, 1939) and used with success by several workers (Trethewie, 1938; Sykowski, Hasekas and Himwich, 1939; Emmens, MacIntosh and Richter, 1942; Feldberg, 1945). To obtain with this method optimal conditions of synthesis incubation should be carried out at 37°C under aerobic conditions and the medium should contain a low concentration of glucose and a high concentration of potassium, up to 50  $\mu\text{g}$  acetylcholine are formed per hour by the equivalent of 1 gram of fresh rat's brain.

(2) *Brain pulp or dried brain suspended in a mixture of saline and chloroform or saline and ether respectively*. These media were introduced by Stedman and Stedman (1937, 1939). Originally they incubated their samples at 37°C, but at this temperature their results could not be confirmed by Mann, Tennenbaum and Quastel. Later they worked at room temperature and Mann et al. showed that the yield of acetylcholine in these media is much greater at room than at body temperature. The effect of temperature has been examined further by Feldberg (1945) using dried brain. An increase in temperature from 20 to 37°C accelerates the synthesis but has the additional effect of inactivating the enzyme system more quickly, so that synthesis comes to an end. Therefore when synthesis is studied for periods longer than a few minutes the yield is much greater at 20°C than at 37°C. Although the oxygen uptake of dried brain is extremely small it synthesizes acetylcholine, when shaken in an ether-saline mixture, only in the presence of oxygen; under anaerobic conditions synthesis comes to an end after a short time, some initial synthesis occurs however also in anaerobic conditions. When dried brain is suspended in ether without saline no synthesis occurs; on the other hand when the volume of saline used is too great a condition is reached in which a water-soluble substance necessary for the synthesis is diluted beyond a point sufficient for the synthesis to proceed. With the dried powder from the guinea-pig's cortex the formation of acetylcholine per hour was 72  $\mu\text{g}$  per gram powder which corresponds to about 16  $\mu\text{g}$  per gram fresh weight. The effect of ether was also obtained when its vapour was bubbled through a suspension of brain pulp in diluted plasma. Mann, Tennenbaum and Quastel explained the accelerating effect of ether and chloroform by their ability to facilitate the release of acetylcholine, thus affecting synthesis indirectly, because they found that the store of acetylcholine held in the tissue decreased in the presence of ether. However, the tissue particles of dried brain powder are able to build up their store

of acetylcholine when suspended in an eserinated saline-ether mixture. This observation does not exclude the possibility that ether, by its lipid solvent action, facilitates release of acetylcholine, but synthesis itself is probably also affected directly.

(3) *Homogenised brain and saline extracts of acetone dried brain*. Nachmansohn and Machado (1943) found that homogenised brain synthesizes acetylcholine anaerobically in the presence of adenosinetriphosphate (ATP) and fluoride, the fluoride being necessary to protect the ATP against phosphatase contained in homogenised brain. This discovery attributes a completely new rôle to ATP. Their main results have been confirmed (Feldberg and Mann, 1944, 1945a). According to Nachmansohn and Machado homogenised rat's brain synthesized under optimal conditions, i.e., in the presence of choline and a high concentration of KCl, 35 to 100  $\mu\text{g}$  acetylcholine per gram, even higher values were obtained with frog's brain. The values may be somewhat too high as their method of assaying acetylcholine on the frog's rectus muscle does not take into account the fact that tissue extracts and subthreshold doses of choline sensitize the muscle to the action of acetylcholine. Feldberg and Mann found that homogenised brain incubated aerobically but under otherwise similar conditions, formed small amounts of acetylcholine only. Better results were obtained with saline extracts prepared from acetone dried and powdered brains. The acetone treatment did not apparently affect the enzyme system, which could be completely separated from the particulate matter of the brain by the saline extraction. Under anaerobic conditions and in the presence of ATP, fluoride and a high concentration of KCl, an extract from 1 gram powder formed in one hour up to 400  $\mu\text{g}$  acetylcholine, this corresponds to about 70  $\mu\text{g}$  per gram fresh weight and to about 1400  $\mu\text{g}$  per gram dry material contained in the saline extract. Under aerobic conditions half these amounts only were formed. The inhibitory action of iodoacetate, copper ions and iodine on the synthesis of acetylcholine led Nachmansohn and Machado to postulate the presence of active SH-groups in the enzyme. Feldberg and Mann suggested that their oxidation to inactive -SS-groups could explain the inhibitory effect of oxygen. It was shown that in the presence of SH-compounds, such as reduced glutathione or cysteine, which would prevent or reverse the oxidation of the SH-groups in the enzyme, the aerobic synthesis of acetylcholine was brought to the same level as, or even to a higher level than, that observed anaerobically. These SH-compounds had some activating effect also on the anaerobic synthesis which suggested that some SH-groups in the enzyme system might have undergone oxidation in the course of the preparation or extraction of the acetone powder. The corresponding -SS-compounds, oxidised glutathione and cystine, inhibited the synthesis of acetylcholine both under aerobic and anaerobic conditions. It thus appears that ATP and SH-compounds or similarly acting substances are essential for the aerobic synthesis of acetylcholine. Unlike the homogenised brain tissue, the extracts prepared from the acetone powder liberated ortho-phosphate from ATP only very slowly. No relation was found between the rate of synthesis of acetylcholine and the rate at which free phosphate was split off. The function of ATP appears to be specific, even closely related derivatives such as adenosinediphosphate (ADP) or inosinetriphosphate were found to be poor substitutes of ATP. It is too early to state precisely which part of the ATP molecule is involved in the formation of acetylcholine. The evidence available so far suggests that the most likely change is the conversion of ATP to ADP (Feldberg and Mann, 1945).

Recently Feldberg and Mann (1945b) showed that ATP is not an irreplaceable component of the synthesizing system. An equally large formation of acetylcholine could be obtained both aerobically and anaerobically if, instead of ATP, citrate and boiled saline extract prepared from acetone dried brain were added to the synthesizing medium. If all three activators, ATP, citrate and boiled juice were added together to the brain extract, the rate of formation of acetylcholine per hour rose to 1200  $\mu\text{g}$  (rat) and 1800  $\mu\text{g}$  (guinea-pig) per gram acetone powder i.e. to over 4 and 6 mg acetylcholine respectively per gram dry material contained in the saline extract.

*Some factors which influence the synthesis* *Oxygen, cyanide, azide* The apparently antagonistic action of oxygen in the different conditions under which the synthesis of acetylcholine has been studied has been pointed out. Quastel, Tennenbaum and Wheatley (1936) assumed that it was closely linked with cell respiration. There is no doubt that when the synthesis is studied in brain slices or pulp, incubated in a saline medium, it is abolished under anaerobic conditions and greatly inhibited by KCN under aerobic conditions. KCN as well as azide however, has no effect on synthesis under conditions where cell respiration is absent, e.g., when dried powdered brain, homogenised brain or saline extracts of acetone brain powder are used (Nachmansohn and Machado, 1943, Feldberg, 1945, Feldberg and Mann, 1944, 1945). It is possible that the respiration in brain slices and pulp is associated with the mechanism of the release of acetylcholine and so affects the synthesis indirectly. The fact, however, that a synthesis of acetylcholine occurs without oxygen, i.e., under conditions where cell respiration is absent, does not prove that oxygen is not necessary for one of the various reactions involved in the synthesis as it occurs in the living tissue. The rôle of oxygen for the synthesis in this condition is all but clear. It may well be that the synthesis *in vivo* occurs in two stages, the first anaerobic, being dependent on the presence of ATP, the second, aerobic, on the presence of oxygen. Such a possibility is not too remote when it is remembered that metabolic processes, such as for instance the carbohydrate metabolism in muscle, occur in two successive stages, anaerobic reactions being followed by aerobic reactions.

*Choline and acetate* Stedman and Stedman (1937) considered choline as the precursor of acetylcholine but they found that it did not influence the synthesis in brain pulp when added to their chloroform-saline medium. Brown and Feldberg (1937), in experiments on sympathetic ganglia, found, although not regularly, that choline exerted a strong accelerating effect on synthesis and such an effect was observed regularly by Mann, Tennenbaum and Quastel (1938) in their experiments with respiring brain tissue. A much stronger accelerating action by choline was observed by Nachmansohn and Machado (1943) on the anaerobic synthesis of acetylcholine in homogenised brain in the presence of ATP. They therefore termed the enzyme responsible for the synthesis "*choline acetylase*". Their results with choline have been confirmed both for the anaerobic and for the aerobic synthesis in homogenised brain and in saline extracts of acetone dried brain. The substrate from which choline is derived is unknown. It may be present as free choline. On the other hand, it may be, as suggested by v. Murali (1943), that phosphatides like lecithin or sphingomyelin yield choline provided that the phosphoryl part is taken up by phosphate acceptors such as aneurin or adenylic acid. These acceptors would be phosphorylated to coenzyme and ATP respectively. There is as yet no conclusive experimental evidence for this theory. Nothing is known about the origin of the acetate group. Nachmansohn, Cox, Coates and Machado (1943) suggest that acetylphosphate or phosphorylcholine may be intermediary products. Sodium acetate had no effect on the synthesis under whatever conditions it was studied (Stedman and

Stedman, 1937, Mann, Tennenbaum and Quastel 1938, Kahlson and MacIntosh, 1939, Nachmansohn and Machado, 1943, Feldberg and Mann, 1944, 1945a) Acetoacetate was suggested by Stedman and Stedman (1937, 1939) as an esterifying agent for choline, as it increased synthesis by brain pulp incubated in a saline-chloroform or saline-ether medium, but it had no effect of this kind on the synthesis by brain pulp incubated in a saline medium or on the synthesis in intact sympathetic ganglia. Acetaldehyde was also without effect (Mann, Tennenbaum and Quastel, 1939, Kahlson and MacIntosh, 1939). As pointed out already, in saline extracts from acetone dried brain, citric acid has a particularly strong activating influence on the formation of acetylcholine. Thus the possibility arises that citric acid also may act as the source of the acetyl groups.

*Glucose and other sugar derivatives* Glucose may accelerate or inhibit synthesis according to the condition under which it is used. It stimulates and is in fact essential for the aerobic synthesis of acetylcholine in brain slices or pulp incubated in a saline medium and this effect is inhibited by d,l-glyceraldehyde (Mann, Tennenbaum and Quastel, 1938, 1939). The optimal concentration for the stimulating action of glucose lies far below the blood sugar level. It is thus not surprising that the effect is not evident when glucose is added to large quantities of brain tissue incubated in a small volume of saline, because the natural glucose, or substances derived from the tissue and acting like glucose, are not sufficiently diluted to create a deficiency which added glucose might relieve. The failure of Stedman and Stedman (1939) and of Sykowsky, Fazekas and Himwich (1939) to observe an increased rate of synthesis under conditions apparently similar to those used by Mann et al. can be explained on these lines (Mann, Tennenbaum and Quastel, 1939, Feldberg, 1945). According to Mann et al., mannose, pyruvate and lactate acted like glucose, galactose had a weak stimulating action and the effect of fructose was doubtful. Hexosediphosphate as well as  $\alpha$ -glycerophosphate did not accelerate the synthesis when brain pulp was incubated, but did so when brain slices were used. Glucose had only a slight stimulating effect on the synthesis in brain pulp suspended in an ether-saline medium and it exerted no stimulating action when dried brain or saline extracts obtained from acetone powder of brain were used. These differences may be explained on the assumption that the stimulating action of glucose is linked with that reaction in the synthesis of acetylcholine which is dependent upon the tissue respiration, the effect cannot be due solely to increased release of acetylcholine, because in the absence of glucose nervous tissue appears to be unable to replace the acetylcholine released by nervous impulses, as was shown by Kahlson and MacIntosh (1939) in their experiments on perfused sympathetic ganglia.

Concentrations of glucose as high or higher than those normally present in blood were found to inhibit synthesis in brain pulp incubated in a saline medium (Feldberg, 1945). It is possible that this effect is due to a direct action of glucose on the release of acetylcholine, and that the synthesis is influenced only indirectly. Glucose has no inhibitory action on synthesis in brain pulp suspended in an ether-saline medium or when dried powdered brain is used, but pyruvate,

according to Stedman and Stedman (1937, 1939) inhibited synthesis in brain pulp suspended in a chloroform-saline medium. Glucose, fructose (but not sucrose) and certain phosphohexoses were found, even in small concentrations, to inhibit strongly the anaerobic as well as the aerobic synthesis in saline extracts of acetone powder in the presence of ATP (Feldberg and Mann, 1944, 1945). The effect could be attributed to an enzymic esterification of the labile phosphate groups of ATP. There is as yet no evidence that this mechanism accounts also for the inhibiting action which large concentrations of glucose have on the formation of acetylcholine in brain pulp. If instead of ATP, boiled juice from brain and citrate are used, the saline extract from acetone dried brain synthesizes acetylcholine equally well in the presence and in the absence of glucose (Feldberg and Mann, 1945b).

*Potassium and calcium* Potassium accelerates and calcium diminishes the formation of acetylcholine in respiring brain slices or pulp. The action of the ions was thought to be one on cell permeability, i.e., on release of acetylcholine and not on synthesis proper. Potassium in fact was found to decrease the acetylcholine content of the tissue slices, but this result needs confirmation. Rubidium and caesium acted like potassium (Mann, Tennenbaum and Quastel, 1938, 1939). When dried brain powder was used, potassium affected the synthesis of acetylcholine only in a saline suspension and not in an ether saline medium, whereas the inhibitory effect of calcium occurred in both media. Potassium also had no effect on the acetylcholine store held in the tissue particles of dried brain suspended in ether-saline, whereas calcium prevented this store from being built up, a fact which suggested that calcium at least acted on the synthesis proper. The finding of Nachmansohn and Machado (1943) that potassium did not accelerate anaerobic synthesis in homogenised brain could not be confirmed by Feldberg and Mann (1944, 1945a). They found that potassium increases and calcium decreases both the anaerobic and the aerobic synthesis of acetylcholine in homogenised brain or in saline extracts of acetone dried brain in the presence of ATP, that is, under conditions where the factor of release of acetylcholine from tissue particles is excluded. A study of the action which Ca and K have on the metabolism of ATP revealed the fact that the Ca ions inhibit the breakdown of ATP to inorganic phosphate whereas K-ions do not affect the dephosphorylation of ATP. Thus the antagonistic action of the two ions is not solely dependent on the manner in which they affect the dephosphorylation of ATP, there must be yet another process connected with the synthesis of acetylcholine in which the two ions are involved.

The release of acetylcholine from cholinergic nerve fibres is generally regarded as the result of a "mobilization of K-ions," which occurs during the passage of a nerve impulse, the release of acetylcholine is followed by its resynthesis, a process said to be independent of the passage of the impulse. There is no doubt that the nerve impulse releases acetylcholine because the tissue store of acetylcholine can be decreased by nerve impulses when synthesis is prevented, as seen in experiments by Kahlson and MacIntosh (1939) on perfused sympathetic ganglia.

The fact that K-ions are involved in the formation of acetylcholine, however, suggests that the "mobilisation of K-ions" also has a direct effect upon the resynthesis. When the synthesis of acetylcholine is studied in particulate matter of brain tissue potassium ions apparently accelerate the synthesis and facilitate the release of acetylcholine, calcium ions, on the other hand, inhibit both synthesis and release.

*Mg and NH<sub>4</sub> ions* Mg-ions and large concentrations of NH<sub>4</sub>-ions inhibit synthesis in respiring brain slices (Mann, Tennenbaum and Quastel, 1939) but have no action of this kind on synthesis in homogenised brain or in saline extracts of acetone dried brain (Nachmansohn and Machado, 1943, Feldberg and Mann, 1944, 1945a).

*Amino and other acids* The anaerobic synthesis of acetylcholine by homogenised brain in the presence of ATP diminishes greatly after dialysis, some reactivation occurs with citric and glutamic acids and to a smaller degree with glutamine, alanine, methionine and succinic acid (Nachmansohn, John and Waelsch, 1943). In saline extracts from acetone dried brain citrate strongly activates the synthesis of acetylcholine. Citric acid can not be replaced by succinic, fumaric or tartaric acids, but malonic, glutamic, and aconitic acid show a slight activating effect. Malonic acid does not inhibit the action of citric acid (Feldberg and Mann, 1945a). In intact sympathetic ganglia or in respiring brain slices succinate and -ketoglutarate are without effect on the synthesis of acetylcholine (Kahlson and MacIntosh, 1939, Mann, Tennenbaum and Quastel, 1939).

*Inhibitors* Cu-ions, iodine and iodoacetic acid inhibit synthesis (Nachmansohn and Machado, 1943, Feldberg, 1945, Feldberg and Mann, 1945a). The effect of iodoacetic acid was observed with brain pulp incubated in saline solution, with dried brain suspended in an ether-saline medium, in homogenised brain and in saline extracts of acetone dried brain, the effect of Cu-ions under the last two conditions and the effect of iodine in homogenised brain. The anaerobic and even more the aerobic synthesis in saline extracts of acetone dried brain in the presence of ATP was inhibited also by ascorbic acid and by hydroquinone (Feldberg and Mann, 1945c).

*Vitamin B<sub>1</sub>, cocarboxylase* Mann and Quastel (1940) found that brain slices from vitamin B<sub>1</sub>-deficient pigeons, incubated in a saline medium containing glucose, pyruvate and a high concentration of KCl, formed less acetylcholine than brain slices from normal pigeons incubated similarly. The synthesis in the brains from the vitamin-deficient pigeons could be restored to normal by the addition of cocarboxylase to the medium. Cocarboxylase had no effect on the synthesis in brain slices of normal pigeons. According to Torda and Wolff (1944), however, both cocarboxylase and vitamin B<sub>1</sub> in low concentrations slightly accelerate and in high concentrations inhibit, aerobic synthesis of acetylcholine in a saline suspension of homogenised frog's brain. On the other hand, neither cocarboxylase nor aneurin are able to replace ATP in the synthesis of acetylcholine in the saline extract of acetone dried brains. In fact, vitamin B<sub>1</sub> was

found to inhibit the anaerobic synthesis in the presence of ATP (Feldberg and Mann, 1945a)

*Adrenaline* increases the aerobic synthesis obtained in a saline suspension of homogenised frog's brain (Torda and Wolff, 1944)

*Quinine* according to Sykowsky, Fazekas and Himwich (1939) increases the rate of synthesis in respiring rat's brain slices

*Stability of the enzyme* Synthesis is greatly diminished or lost when intact brain, brain pulp or homogenised brain is kept frozen for several days (Nachmansohn and Machado, 1943, Feldberg, 1945) Brain dried in the desiccator or acetone dried brain, however, retain their ability to synthesize acetylcholine when kept in the cold (Feldberg, 1945, Feldberg and Mann, 1945)

*Synthesis of acetylcholine in different parts of the central nervous system and in different species* Some caution is necessary when comparing the synthesis in different tissues For instance, rat's brain synthesizes large amounts of acetylcholine when slices are incubated in a saline medium or when the homogenised brain or saline extracts of the acetone dried brain are incubated in the presence of ATP, but only small amounts are formed when dried powdered brain is suspended in an ether-saline medium The evidence so far available suggests that synthesis does not increase with the phylogenetic development, on the contrary the highest rate of synthesis so far observed has been with frog's brain (Nachmansohn and Machado, 1943) and cat's, dog's and monkey's brain were found to be less active than rat's and guinea pig's brain The activity in different parts of the central nervous system decreases in the following order cerebral cortex, brain stem, medulla, spinal cord, cerebellum (Feldberg, 1945) As the cerebellum is relatively rich in cells, synthesis is not simply related to the density of cells in the tissue Although no data are available on the synthesis in various finer structures of the central nervous tissue comparable with those on the acetylcholine content, there appears to be a relation between the two Those parts which are relatively rich in acetylcholine synthesize relatively large amounts of acetylcholine The statement of Sykowsky, Fazekas and Himwich (1939) that brains of new born rats synthesize acetylcholine at a greater rate than those of adult rats, which appears to contradict this conclusion, needs confirmation The acetylcholine content of motor roots is much higher than that of any part of the central nervous system, but the ability of brain tissue of rats and guinea pigs to synthesize acetylcholine appears to be as high, if not higher, than that of motor roots (Feldberg, 1943, Feldberg and Mann, 1945c) Too few experiments have yet been carried out on the synthesis of acetylcholine in peripheral nervous tissue to allow a comparison with the synthesis in central nervous tissue There is no strict parallelism between the choline esterase content of nervous tissue and its ability to synthesize acetylcholine, whereas there is a close relation in some tissues there is gross discrepancy in others At present, experiments are lacking in which the same author has compared, in various nervous tissues, the acetylcholine content the cholinesterase content and the ability to synthesize acetylcholine under different conditions



**6 RELEASE OF ACETYLCHOLINE** Only results obtained on intact tissue will be considered here, those obtained on brain slices, pulp or powder have been dealt with in the previous section. The evidence available so far suggests that stimuli which are effective in slices, pulp or powder are also effective on intact nervous tissue.

*Spontaneous release* The central nervous system exhibits continuous activity and on the hypothesis of central synaptic transmission by acetylcholine, its continuous release in the absence of any external stimuli is to be expected. In fact, acetylcholine appears in the cerebrospinal fluid of dogs and cats after intravenous injections of eserine or when the ventricular system is perfused with eserinated solutions (Feldberg and Schriever, 1936, Chang, Hsieh, Li and Lim, 1938, Adam, McKail, Obrador and Wilson, 1938). The concentration of acetylcholine in the C.S.F. rose with the dose of eserine injected intravenously. This may be due to the fact that a more complete inhibition of the cholinesterase activity leads to a greater accumulation of acetylcholine and consequently to increased activity of the central nervous system. Chang, Chia, Hsu and Lim (1937) detected acetylcholine in the venous blood from the isolated dog's head *vivi*-perfused by a second eserinated dog. Chute, Feldberg and Smyth (1940) observed the output of small amounts of acetylcholine when they perfused the almost completely isolated cat's brain with 50 per cent defibrinated eserinated blood, and Bülbring and Burn (1941) made a similar observation when perfusing the spinal cord of dogs. The fact that Altenburger (1937) was unable to detect acetylcholine in human C.S.F. is probably to be explained by inability to inject into patients sufficiently large doses of eserine.

*Potassium* causes temporarily an increase in the small spontaneous output of acetylcholine from the perfused cat's brain (Chute et al., 1940).

*Adrenaline* causes the appearance of acetylcholine or an increase of its concentration in the cerebrospinal fluid of eserinated dogs (Feldberg and Schriever, 1936, Chang et al., 1938). The effect also occurred but with some delay when the pressor effect of adrenaline was prevented by the use of a compensator. In the experiments of Adam et al. (1938) adrenaline was ineffective. Adrenaline probably does not release the acetylcholine directly, a possibility discussed by Bonvallet and Minz, (1938) but lowers the threshold for the transmission of impulses within the central nervous system. This will result in an increased spread of a given sensory impulse and thus to increased release of acetylcholine (Bülbring and Burn, 1941).

*Pituitrin* According to Adam et al. (1938) very large concentrations of pituitrin in the fluid used for perfusing the ventricular system double the acetylcholine concentration of the outflowing fluid. Pituitrin given intravenously did not have this effect.

*Asphyxia* causes the appearance of acetylcholine, or its increase, in the cerebrospinal fluid of eserinated dogs. The effect is obtained after removal of the suprarenal glands and is independent of the rise in arterial blood pressure (Feldberg and Schriever, 1936). Adam et al. (1938) found no such effect in dogs on breathing a mixture of 7 per cent carbon dioxide in air.

*Direct stimulation* Strong faradic stimulation of the isolated cord of rabbits (Mins, 1936) and of the brain-cord preparation of toads (Li, 1938) caused the release of acetylcholine into the surrounding fluid. According to Li a constant current passing through the braincord preparation did not have this effect. The acetylcholine-like activity which, according to Blinova and Lobova (1936), appears sometimes in the cerebrospinal fluid and in the venous blood collected from the sinus after stimulation of the cerebral cortex cannot be due to acetylcholine because the dogs used for these experiments received no eserine. Adam et al. (1938) when perfusing the ventricular system with eserinated Locke solution, obtained no increase in the output of acetylcholine by stimulation of the motor or cerebellar cortex or of the spinal cord, but after stimulation of the hypothalamus the acetylcholine concentration in the perfusate rose to about 100 per cent in half the experiments. This may be associated with the release of acetylcholine from nervous connections to the posterior pituitary (see section 8).

*Stimulation of afferent nerves* During central vagus stimulation Dikshit (1934) sometimes observed the appearance of acetylcholine in the cerebrospinal fluid of cats and Bykow (1935) and Benetato and Munteanu (1936) in the blood leaving the medulla. Feldberg and Schriever (1936) were unable to confirm the results of Dikshit in dogs. Negative results were obtained also by Adam et al. (1938) in their perfusion experiments on the ventricular system, when they stimulated the vagus centrally or other afferent nerves. According, however, to Chang and his co-workers (Chang, Chia, Hsu and Lim, 1937, 1938, Chang, Lim and Lu, 1938) stimulation of the central end of the vagus or of the sinus nerve leads to the appearance of acetylcholine in the venous blood from the isolated dog's and cat's head, *vivi* perfused by a second animal. The effect is not wholly due to the release of acetylcholine from nervous connections with the posterior pituitary, as a delayed effect occurs also on hypophysectomised animals. In later experiments Chang, Hsieh, Li and Lim (1938) succeeded in demonstrating the appearance of acetylcholine, or its increase, in the cerebrospinal fluid after central vagus stimulation when eserine had been given not only intravenously, as in the experiments of Feldberg and Schriever, but also intraspinally. There is thus good evidence that central vagus stimulation leads to the release of acetylcholine. Bülbürg and Burn (1941) found that, when they perfused the lower half of the spinal cord with eserinated saline solution instead of blood, reflex activity persisted for about 8 minutes. During this period stimulation of the central end of the cut sciatic nerve caused the appearance of acetylcholine (1 in 50 millions) in the venous effluent.

7 ENZYMATIC HYDROLYSIS OF ACETYLCHOLINE. Although it is possible that part of the released acetylcholine may be reconverted into its inactive form in the tissues, most of it is probably destroyed at once by cholinesterase. This conclusion is based not only on the presence of cholinesterase in the central nervous tissue but also on the direct evidence that when cholinesterase is inhibited by eserine or other anticholinesterases, and only then, does acetylcholine appear in the cerebrospinal fluid or in the venous blood, and that the accumulation of

acetylcholine under this condition produces the central actions expected from the pharmacological activity of eserine

Dale, as early as 1914, postulated the presence of an enzyme in blood able to hydrolyse acetylcholine and responsible for the evanescent character of its response after intravenous injection. Loewi and Navratil (1926) showed that tissue extracts destroyed acetylcholine and stressed the enzymatic nature of the process. Galehr and Plattner (1927) demonstrated the quick destruction of acetylcholine by blood but thought that no enzymatic process was involved in the reaction. Its enzymatic nature, however, was proved by Engelhart and Loewi (1930) and by Matthes (1930). Stedman, and Stedman and Easson (1932) demonstrated the specificity of the enzyme responsible for the destruction of acetylcholine in blood and named the enzyme cholinesterase. Further proof for the specificity was given by Easson and Stedman (1937). The Stedmans and their co-workers, however, pointed out that blood cholinesterase behaved somewhat differently in different species. The possibility of the existence of different cholinesterases has been considered subsequently by different authors (Hall and Lucas, 1937, Alles and Hawes, 1940, Richter and Croft, 1942). Mendel and Rudney (1942) maintain that the cholinesterase present in serum and certain tissues is non-specific and they call it pseudocholinesterase. De Laubenfels (1943) and Alles and Hawes (1944) think it inadvisable to give this name to the serum enzyme. A different cholinesterase, acting exclusively on choline esters, is present according to Mendel and Rudney in brain tissue and in the red blood cells of some animals and is particularly effective at low concentrations of acetylcholine, high concentrations of acetylcholine inhibit its activity. Some caution therefore is necessary when results obtained with serum cholinesterase are applied to brain cholinesterase.

Stedman and Stedman (1935) were the first to show the presence of relatively large amounts of cholinesterase in brain tissue and its absence in cerebrospinal fluid. According to Altenburger (1937) the destruction of acetylcholine in human liquor proceeds about 250 times more slowly than in blood. The Stedmans found that the concentration of cholinesterase in the basal ganglia of cats is about twice that in the cortex. In table 2 the distribution of cholinesterase in the central and in the peripheral nervous system has been tabulated from the papers of Nachmansohn, 1937, 1938a, b, Martini and Torda, 1938, Nachmansohn, 1939a, Couteaux and Nachmansohn, 1940, Nachmansohn and Meyerhof, 1941, Nachmansohn and Hoff, 1944. The following conclusions may be drawn

(1) The cholinesterase concentration is greater in grey than in white matter. But not every region rich in cells yields a high concentration.

(2) There is no increase in the cholinesterase concentration with the phylogenetic order of the species. In fact the reverse is true. The highest value was obtained with the head ganglion of the squid and some of the lowest values were obtained with human cortex.

(3) A comparison with the peripheral nervous system shows that some regions of the C N S, although rich in cells, do not contain a greater concentration of cholinesterase than peripheral nerves and that sympathetic ganglia of cats have a higher concentration than nearly all parts of the central nervous system of mammals.

TABLE 1  
*Acetylcholine content of nervous tissue*

ANIMAL	TISSUE	MG. ACETYLCHOLINE PER GRAM FRESH WEIGHT
Central nervous system		
Ox	Optic nerve and chiasma	0
Man	Cortex white matter medulla cerebellum	0.1-0.3
Cat	Cerebellum optic nerve	
Man	Corpus striatum crux cerebri spinal cord	0.2-0.6
Dog	Cortex white matter medulla pons cerebellum	
Cat	Occipital lobe white matter dorsal columns pyramids	
Water buffalo	Cortex white matter medulla pons corpus striatum cerebellum	
Dog cat	White matter	0.2-1
Man	Thalamus	0.6-1
Dog	Spinal cord optic chiasma	
Water buffalo	Cortex thalamus	
Dog	Whole brain	0.5-2
Rabbit	Whole brain cortex	
Cat	Internal capsule	0.2-3.3
Dog	Corpus striatum crux cerebri, thalamus spinal cord	1-2.7
Cat	Medulla pons midbrain olf bulb hypo- thalamus	
Rabbit	Cortex	
Man	Basal ganglia	1.5-3
Cat	Corpus striatum	
Rat mouse	Whole brain	
Pigeon	Whole brain	
Cat	Cortex corpus callosum	0.9-4.5
Cat	Thalamus whole brain	1.5-4.5
Dog	Grey matter	
Cat	Region of III nucleus pons superficial medial	2.5-5
Rabbit	Brain stem	
Guinea pig	Whole brain	
Frog	Spinal cord	
Ox	Retina	5-6

TABLE 1—*Concluded*

ANIMAL	TISSUE	$\mu$ G ACETYLCHOLINE PER GRAM FRESH WEIGHT
<i>Central nervous system—Concluded</i>		
Cat	Basal ganglia	7
Frog	Whole brain	5-10
Octopus	Optic ganglion	77
<i>Peripheral nervous system</i>		
Dog, cat	Posterior roots	0.0-25
Ox	Postganglionic sympathetic fibres	0.6-15
Cat	Somatic mixed nerves	2.5-7
Cat	Vagus, nodose ganglion	6-9
Dog	Ventral lumbar roots	9-10
Dog	Sympathetic cholinergic nerves	6-14
Cat	Somatic nerves, mainly motor	6-16
Cat	Ciliary ganglion	12
Sheep	Sympathetic ganglia	8-19
Cat	Anterior roots	12-22
Sheep	Sympathetic cholinergic nerves	12-23
Cat	Sympathetic ganglia	10-44
Cat	Sympathetic cholinergic nerves	18-40

(4) A comparison with the distribution of acetylcholine in central nervous tissue, as shown in table 1, does not yield a great deal of information owing to the absence of sufficient parallel estimations of the two components. Some parts rich in cholinesterase are relatively rich also in acetylcholine. The two values agree fairly well with regard to white and grey matter, to species differences, to thalamus and basal ganglia and to sympathetic ganglia. On the other hand, the cerebellum which is relatively rich in cholinesterase contains very little acetylcholine and the motor roots show a discrepancy in the opposite direction. Hellauer (1939) used a different method of determining the cholinesterase concentration in nervous tissue and found good agreement between acetylcholine and cholinesterase content in rabbit's spinal cord and frog's C N S but not in the peripheral nervous system.

When evaluating the figures given in table 2 some caution is necessary. According to Hellauer (1939) the distribution of cholinesterase differs from that

TABLE 2  
*Cholinesterase activity of different parts of the nervous system*  
Expressed in milligram acetylcholine hydrolysed by 100 mgm. finely divided tissue in 1 hour

Central nervous system		
White matter, brain	(ox, dog)	0.25-0.34
Spinal cord, cervical bulb	(rat)	0.24-0.43
Spinal cord, lumbar bulb	(rat)	0.33-0.48
White matter, spinal cord	(cat)	0.6
Cortex	(man)	1.2
Optic nerve	(dog)	1.6
Cortex	(dog ox)	2-3
C.N.S.	(electric fish)	1-4
Thalamus	(man)	2.7
Thalamus	(ox, dog)	2-5
Olf. bulb	(rabbit)	4
Post. quadrig. bodies	(ox)	4
Spinal cord	(ox rat)	5
Brain	(rat)	7
Spinal cord	(man dog)	6-9
Cerebellum	(rabbit)	9
Spinal cord	(rabbit)	10
Brain	(rabbit)	11
Ant. quadrig. bodies	(ox)	10-12
Post. quadrig. bodies	(rabbit)	12.5
Retina	(dog ox)	13-20
Spinal cord	(cat)	11-25
Ant. quadrig. bodies	(rabbit)	25
Brain	(chicken)	25
Caudate nucleus	(ox, dog)	23-57
Putamen	(man)	46
Lentiform	(ox)	67-73
Head ganglion	(squid)	260-465
Peripheral nervous system		
Post. root	(dog)	1.4
Sciatic nerve	(dog)	1
Ant. roots	(dog)	2.9
Spinal gangl.	(dog)	3
Sympath. gangl.	(dog)	11-19
Sympath. gangl.	(cat) (N 1938, b)	18-21
Sympath. gangl.	(cat) { C & N 1940	{ 40-60
Denerv. sympath. gangl.	(cat) }	{ 20-25

shown in table 2. He found a much greater difference in the cholinesterase concentration of posterior and anterior roots, the values for the latter (dog) were

in fact about 60 per cent higher than those for rabbit's spinal cord. In addition, he determined the cholinesterase content in the central nervous system of frogs and found it to be about 10 times higher than that of rat's spinal cord and about 6 times higher than that of dog's anterior roots, so that it would assume either the value of 50 or of 18 in table 2. Even in the hands of the same author different values are obtained for the same tissue. The value for the sympathetic ganglion varied more than 100 per cent, in 1938 Nachmansohn found it to be lower than after denervation in 1940 (see table 2).

On the other hand there is a definite relation between cholinesterase concentration and functional development of the different parts of the central system. In brains of chick embryos, which are fairly well developed at hatching, the concentration of cholinesterase rises during the last four days before hatching. In rabbits and rats the rise occurs during the first three weeks after birth when the brain develops (Nachmansohn, 1938a, b). In the sheep's foetus the cholinesterase is already high in the early stages of gestation in the spinal cord but rises in the brain, the functional development of which occurs later, in the last weeks of gestation (Nachmansohn, 1940). Similarly Youngstrom (1941) found a good relation between the order of morphological development of the different parts of the central nervous system of the human foetus and the increase in cholinesterase concentration.

Martini and Torda (1938) found in rats that the cholinesterase activity of the spinal cord diminished two days after transverse cord section in the parts distal to the section, but returned nearly to normal within 2 to 3 weeks with the return of reflex activity. After cutting and degeneration of the dorsal roots Nachmansohn and Hoff (1944) found in cats a slight decrease in the cholinesterase concentration of the posterior as well as of the anterior horn of the corresponding levels of the spinal cord. The change was small compared with the great individual variations.

8 CHANGES IN THE ACETYLCHOLINE METABOLISM DURING SOME CONDITIONS OF INCREASED AND DECREASED CENTRAL ACTIVITY. Experiments have been carried out in order to discover whether, under acute changes of central activity, changes occur in the acetylcholine content or in the activity of the cholinesterase or of the synthesizing enzyme of the brain tissue. From the results described in section 6 it would appear that nervous tissue is unable to undergo acute changes in its acetylcholine content, but some such changes have been reported. A striking opportunity for attributing the effects of convulsive drugs to inhibition of cholinesterase activity was given by eserine. In this section conditions not yet reviewed in detail will be discussed.

*Convulsions by strychnine, metrazol or tetanus toxin.* The acetylcholine content of the central nervous system of frogs was found to be increased after prolonged strychnine convulsions lasting for several hours (Loewi, 1937, Cortell, Feldman and Gelhorn, 1941). In the experiments of Loewi the content doubled. But no such changes were observed by Cortell et al. in the brains of eserinated rabbits killed after a 5 minutes period of convulsions by strychnine or metrazol. Ac-

According to Fegler, Kowarzyk and Lelusz-Lachowicz (1938) a few minutes of strychnine convulsions decrease and of tetanus convulsions increase the acetylcholine content of the central nervous system of rabbits. Their method of extraction, however, does not determine the acetylcholine content of the tissue and the meaning of the results is obscure. Large concentrations of strychnine were found to inhibit cholinesterase activity (Ammon, 1934, Nachmansohn, 1938b). According to the *in vitro* experiments of Nachmansohn, strychnine concentrations of 1 in 25,000 and of 1 in 7,000 inhibit about 20 and 60 per cent respectively. The concentration of strychnine in the grey matter of the spinal cord is several times lower, about 2-6  $\mu\text{g/g}$  (Veit, 1935), so that it is doubtful if in this condition strychnine exerts any inhibitory action on the cholinesterase. In fact no changes were found by Pighini (1939) in cholinesterase activity of brains of dogs and rabbits during strychnine or tetanus convulsions and by Cortell et al in brains and spinal cord of frogs after 4 hours of strychnine convulsions, despite the increased acetylcholine content of the tissue.

**Insulin convulsions** No change has been observed in the acetylcholine content and cholinesterase activity of brain tissue of mice, rats or rabbits during hypoglycemic shock (MacIntosh, 1939, Mann, Tennenbaum and Quastel, 1939, Cortell, Feldman and Gelhorn, 1941). Welsh (1943) found that mincing rat's cortex in ice cold aerated saline solution brought less acetylcholine into solution when the cortex was from a rat killed during hypoglycemic shock. His claim, however, to have demonstrated that insulin hypoglycemia causes a decrease in the level of acetylcholine in the central nervous system is not borne out by these results. The ability of minced brain incubated in a saline medium to synthesize acetylcholine was found to be the same when the brains were from normal or insulin convulsed rats (Mann, Tennenbaum and Quastel, 1939). A new suggestion has been made by Feldberg (1945) to explain the insulin convulsions, based on the observation (see section 5) that glucose, in concentrations present in blood, inhibits the synthesis or the release of acetylcholine in minced brain. According to this view the blood glucose would normally exert a restraining effect on the continuous release or synthesis of acetylcholine in the central nervous system, lowering the blood sugar would remove the glucose "brake" and allow the synthesis and release of acetylcholine to proceed at an abnormal rate. This explanation would account for the failure of previous attempts to correlate the convulsions with acetylcholine. The inhibition by glucose may be due to esterification and thus to inactivation of ATP, which appears to be involved in the synthesis of acetylcholine, or to an effect on the mechanism of its release. The hunger contractions associated with a lowering of blood sugar might be interpreted by a similar mechanism partly in the periphery, partly on the acetylcholine metabolism at the vagal centre.

**Asphyxia,  $\text{CO}_2$**  The results of experiments on the release of acetylcholine by asphyxia and increased  $\text{CO}_2$  pressure of the alveolar air have been given on page 624. Increased  $\text{CO}_2$  pressure was found to have little or no effect on the knee jerk (Schweitzer and Wright, 1937) or on the flexor reflex but depressed the



response to electrical stimulation of the cortex like acetylcholine (McKail, Obrador and Wilson 1939) This effect was readily suppressed by atropine and sometimes potentiated by eserine which is "strong presumptive evidence of the release of acetylcholine in the central nervous system" Since the spinal reflexes were relatively insensitive to  $\text{CO}_2$  the action was probably mainly on the cortex The release of acetylcholine could be effected in one of several ways  $\text{CO}_2$  might increase the sensitivity of the nerve cells to acetylcholine and thus increase the spread of sensory impulses by analogy with the interpretation of the similar action of adrenaline  $\text{CO}_2$  might facilitate the release or accelerate the synthesis of acetylcholine Ether (even as vapour) and chloroform increase the synthesis of acetylcholine in brain pulp *in vitro* and consequently it is possible that some of the excitatory effects of the induction stage of chloroform or ether anesthesia may be accounted for in this way

*Anoxia* Cortell, Feldman and Gelhorn (1941) found no change in acetylcholine and cholinesterase content of rabbit's brain when the animal had been subjected to low oxygen pressure According to Welsh (1943), mincing rat's cerebral cortex in ice cold esermined saline solution brings into solution less acetylcholine than normal when the rat has previously been subjected to low oxygen pressure His claim that anoxia decreases the acetylcholine level of brain is not borne out by his experiments

*Heat coma* Keeping frogs for 15 minutes at  $37^\circ$  to  $39^\circ\text{C}$ , which produces a comatose condition, decreases the acetylcholine content and the cholinesterase activity of the central nervous system (Cortell, Feldman and Gelhorn, 1941)

*Vitamin B<sub>1</sub> deficiency* caused no changes in the acetylcholine content of the cerebral hemispheres or cerebellum of pigeons or the brain of rats (MacIntosh, 1939) Mention has been made of the observation that the synthesis of acetylcholine is impaired in this condition and that cocarboxylase has a restorative effect

*Release of posterior pituitary hormone* There is some evidence that the nerve fibres responsible for the secretion of the posterior pituitary hormone are cholinergic Mention has been made of a relatively high acetylcholine content of the hypothalamic region (table 1) and the release of acetylcholine into the cerebrospinal fluid on electrical stimulation of this region In addition acetylcholine was found to cause secretion of posterior pituitary hormone (Pickford, 1938, 1939) These experiments were initiated by the observation of Chang and his co-workers that a reflex secretion of posterior pituitary hormone occurred on central vagus stimulation and was associated with a release of acetylcholine into the blood stream In hypophysectomised animals this release of acetylcholine was delayed, indicating that in normal animals it was partly associated with the posterior pituitary (Chang, Chia, Hsu and Lim, 1937, 1938, Chang, Lim and Lü, 1938)

*Barbiturates, morphine and apomorphine* Schütz (1943) found that the activity of serum cholinesterase of human blood diminished after prolonged administration of barbiturates Croft and Richter (1943) are reluctant to

attribute definite physiological significance to the changes observed under different conditions in blood serum cholinesterase, since its concentration is usually lower than that of blood corpuscles or of the tissue where most of the destruction of acetylcholine may be presumed to occur. Schütz found, however, that a prolonged administration of barbiturates in animals decreased not only the cholinesterase of serum but also that of the spinal cord and muscle. A single dose of the drugs sufficient to cause deep sleep did not have this effect. When barbiturates are given to epileptics the decrease in serum cholinesterase becomes maximal after three weeks, whereas the number of seizures becomes minimal within a few days, sudden withdrawal of the drug causes the number of seizures to rise sharply to a maximum whilst the cholinesterase is still at a low level, but seizures become less frequent again when the cholinesterase starts to rise (Schütz, 1944).

In *in vitro* experiments phenobarbitone does not inhibit the brain cholinesterase (Bernheim and Bernheim, 1936) which is however inhibited markedly by morphine (1 in 30,000) and apomorphine (1 in 48,000). Bernheim and Bernheim suggest that this action may contribute to some of their stimulating central effects. The common anesthetics inhibit cholinesterase only in much higher concentrations.

Confirmation is needed for the statement of Hoff and Pilcher (1935) that the acetylcholine content of rabbit's medulla, determined by acid alcohol extraction, rises from the normal value of  $0.1 \mu\text{g/g}$  to 10 and even  $100 \mu\text{g/g}$  on stimulation of the periarterial nerves in the wall of the aorta and carotid communis, and also for the statement of Haas (1939) that the acetylcholine content of the brain stem of rabbits, as determined by acid alcohol extraction, increases on intracisternal injection of large doses of *pilocarpine* and *adrenaline* but decreases on intracisternal injection of small doses of *ergotamine*, large doses were ineffective. *Ergotamine* decreased also the acetylcholine content of the cortex. No changes were observed after intravenous injections of these drugs. The observation of Diaz, Lorente, Moran and Simone (1941) that a substance like acetylcholine appears in the urine during attacks of *migraine* also needs confirmation.

Injectations of large "convulsant" doses of acetylcholine have been used in the treatment of epilepsy, schizophrenia and other mental diseases but without success. For references see Cohen, Thale and Tissenbaum (1944).

**9 THE PROPAGATION OF NERVE IMPULSES AND THE RELEASE OF ACETYLCHOLINE FROM CHOLINERGIC NERVE FIBRES** The suggestion has been made that acetylcholine is not only responsible for synaptic transmission but also for the propagated nerve impulse. Such a theory if proved would have a bearing on the chemical theory of synaptic transmission and the evidence in its favour as well as that against it must be included in this review.

Mention has been made of the findings that acetylcholine and cholinesterase are present in nerve fibres (see tables 1 and 2) and that the capacity to synthesize acetylcholine is not limited to the nerve endings but has to be attributed to the whole course of the fibre. Moreover there is evidence to show that the passage

of a nerve impulse releases acetylcholine not only at the endings of cholinergic nerves but also along their whole course, analogous conclusions apply to adrenaline and adrenergic nerves (Calabor, 1933, Bergamin, 1936, 1937, 1938, v Muralt, 1937, Lissak, 1939) What is the physiological significance of these observations? There are three possibilities

(1) No function is associated with the release of acetylcholine or of adrenaline along the fibre

(2) The release of acetylcholine or of adrenaline modifies the nerve impulse

(3) The release of acetylcholine is causally connected with and responsible for the nerve impulse This possibility was envisaged by v Muralt (1937) In a later publication (1943) he leaves open the question whether the release of acetylcholine is responsible for the spike potential or for the after-potentials, i.e., the recovery processes Dale (1938) has stated the fundamental issue involved in this problem as follows "if the liberation of a chemical mediator at a nerve ending should prove to be, not a process peculiar and limited to that ending, but merely a local intensification to ensure transmission to a contiguous cell, of a process which actually figures in the propagation of the impulse along the nerve fibre, we should have to make yet a further revision of our existing conceptions" and the particular difficulty in postulating a complete break in the nature of the processes concerned in conduction along fibres and transmission across synapses would then disappear, "but only at the cost of a more fundamental change of conception concerning the nature of the propagated wave of excitation, than any which has yet been considered seriously" In fact, we should have to assume different mechanisms of nerve propagation for nerves which contain and for those which do not contain acetylcholine (Loewi and Hellauer, 1938, Hellauer, 1939)

The theory that acetylcholine is causally connected with the action potential of nerve fibres has recently been taken up again by Nachmansohn and his co-workers (Nachmansohn and Meyerhof, 1941, Nachmansohn, Coates and Cox, 1941, Nachmansohn, Cox, Coates and Machado, 1942, 1943, Nachmansohn, 1943, Fulton and Nachmansohn, 1943) They base their theory (1) on the presence of a high concentration of cholinesterase in nerves, which in the fibres of the squid is localised in the sheath, and (2) on the close parallelism they found between electromotor force and cholinesterase concentration in the electric organ of fishes Their theory assumes that the discharge from the electric organ and the nerve action potential are fundamentally similar phenomena and that if the one is caused by the depolarising action of acetylcholine the other is initiated by a similar mechanism The following considerations throw doubt on the justification of the interpretation given to their results

(1) Nachmansohn and Meyerhof (1941) compared for the electric organs of the ray, torpedo and electric eel the voltage and number of plates per cm with the concentration of cholinesterase and found a striking parallelism This would mean that each plate has about the same concentration of cholinesterase There are facts, however, which are not easily compatible with this view and suggest that the cholinesterase concentration is not the determining factor for the electromotor force (E M F) but for the rate of subsidence

of the discharge i.e. the disappearance of the released acetylcholine. For instance in the electric organ of the torpedo the action potential of the discharge has a quick descending phase. Eserine does not increase the potential i.e. the E.M.F. is independent of the cholinesterase activity; but eserine lengthens the descending phase (Feldberg and Fessard 1942). Such a lengthened discharge is the normal feature of the discharge from the ray untreated with eserine (Auger and Fessard 1939) suggesting a lower concentration of cholinesterase than in the torpedo. The fact that the calculations of Nachmansohn and Meyerhof do not reveal such a difference makes it doubtful whether we are justified in laying much stress on the parallelism between voltage and cholinesterase concentration.

(2) The parallelism between E.M.F. and cholinesterase concentration is not so strict as it is claimed to be as was shown by those experiments (Nachmansohn, Cox, Coates and Machado, 1942) in which actual determinations of E.M.F., number of plates and cholinesterase concentration were made from the same region of different organs even in the same species of fish. Discrepancies of the order of several hundred per cent were found when cholinesterase concentration was compared with either voltage of the discharge per centimeter or per plate.

(3) As the E.M.F. is dependent on the number, size and form of the plates and as the discharge is induced through nervous action only, it is not surprising that there should be some parallelism between E.M.F. and the concentration of a substance which like cholinesterase is either concentrated at the nerve endings or at the surface of the plates in close proximity to the endings but not with substances which are not concentrated at this active region. Such a parallelism however, does not justify correlating the size of the potential with the concentration of cholinesterase and then applying this parallelism to nerve fibres.

(4) There is no parallelism between the size of the action potential in nervous tissue and the concentration of cholinesterase (see table 2).

(5) The hypothesis that acetylcholine is intrinsically connected with the causation of the action potential in nerves does not take into account the fact that some nerve fibres are free from acetylcholine or contain traces only (see table 1). These fibres are either unable to synthesize acetylcholine or they have this property to a very small degree. If we are thus forced to assume that different substances are responsible for the propagation of nerve impulses in different fibres then the presence of cholinesterase in those fibres which are not cholinergic would prove that its presence is of no special significance for the causation of the action potential. It is worth while remembering the wide distribution of cholinesterase in non nervous tissue emphasized by Torda (1942).

(6) Even in cholinergic nerves impulse conduction and acetylcholine content or the ability to synthesize it do not run parallel. In degenerating nerves the nerve fibres are still able to conduct impulses at a time at which they have lost their acetylcholine content as well as the ability to synthesize it (Feldberg 1943).

(7) Action potentials are not confined to nervous tissue. The action potentials of skeletal muscles would have to be explained on entirely different lines since the muscle fibres contain little acetylcholine or cholinesterase.

(8) There is no evidence that acetylcholine which excites motor endplates, electric plates and nerve cells, probably by a depolarising action, has a similar effect on nerve fibre. Fraser (1937) found no effect of the active principle of the calabar bean on nerve fibres which would warrant such a conclusion. and Cowan (1938) stated that treatment of a frog's nerve with prostigmine or eserine produced practically no change in the action current and only slightly modified the responses of nerve muscle preparations to stimuli of different frequencies. More recently Cantoni and Loewi (1944) have shown that in frogs a subcutaneous injection of eserine did not affect the propagation of impulses in the peripheral nerves despite the fact that their cholinesterase activity was inhibited. Riesser and Nauschloz (1921) obtained no excitation of motor nerves by immersing them in acetylcholine solutions. Bronk (1939) showed that the injection of acetylcholine into a perfused sympathetic ganglion caused a vigorous discharge from the ganglion cells but initiated no impulses in the preganglionic fibres although the presynaptic neurons were accessible to exciting

agents in the perfusion fluid, as shown by their response to sodium citrate. Recently Lorente de No (1944) has shown that acetylcholine even at a concentration of 0.1 M does not depolarise or excite the nerve fibre or prevent the conduction of nerve impulses. In fact whereas acetylcholine may be the sole effective stimulus for the discharge of electric plate being inexcitable to electric or mechanical stimuli, the nerve fibre can be excited by a number of stimuli but apparently not by acetylcholine.

In conclusion it may be said, therefore, that apart from the fact that the nerve impulse releases acetylcholine from cholinergic nerves and may even be associated with its resynthesis, there is at present no evidence to warrant the conclusion of a further intrinsic relationship between nerve impulse and acetylcholine metabolism. It is conceivable that the released acetylcholine may influence the excitability or conductivity of cholinergic fibres and may have some modifying influence on the action potentials. Only further research carried out, not on electric organs but on nerve fibres, can give an answer to this problem. In this connection it is interesting to note that eserine in relatively low concentration affects the membrane potential of nerve fibres during anoxia and during the following period of oxidative recovery (Lorente de No, 1944).

#### CONCLUSIONS

The present position of the theory of acetylcholine as central transmitter is all but settled. The acceptance or rejection of the theory will depend upon the relative value which is to be attached to the various findings reviewed in the foregoing pages and upon what kinds of evidence are regarded as decisive. No reference has been made to the short central synaptic delay or to the short refractory period in considering transmission at central synapses and for the following reason: the shortness of these time intervals does not in itself provide evidence either for or against the theory that acetylcholine is the transmitter substance at central synapses. If there is sufficient other evidence available in support of the acetylcholine theory these time intervals demonstrate only (a) the rapidity with which the acetylcholine is released and acts, which must exclude any process of diffusion from the site of liberation to the site of action, and (b) the rapidity with which the acetylcholine is inactivated within the refractory period, which probably excludes any but enzymatic processes.

All the evidence in favour of acetylcholine as the central transmitter substance has been obtained by methods previously applied to the peripheral nervous system and the arguments in favour of the theory must necessarily be based on the same kind of reasoning as was applied to the peripheral nerve effects. The presence of acetylcholine and of cholinesterase in central nervous tissue, the ability of such tissue to synthesize acetylcholine and to release it under certain conditions, the central effects of acetylcholine and of eserine, all provide strong evidence in favour of the theory that the transmission across a number of synapses in the central pathway of autonomic and motor neurons occurs through the mediation of acetylcholine. In fact the evidence in some instances is as good as or better than that available for the transmission by acetylcholine across ganglionic synapses or from motor nerves to motor endplates. On the other hand there is little evidence in favour of such a mechanism of transmission across

many other central synapses, and there are some facts which are at present difficult to reconcile with the theory of acetylcholine as the universal central transmitter

(1) The acetylcholine content of the brain decreases with the higher development of the species and this decrease can scarcely be attributed to a greater proportion of supporting tissue in more highly developed brains. Nor can it be accounted for by the assumption that a lowering of the store of acetylcholine is associated with increased ability to synthesize it, an explanation which has been put forward to explain the difference in the peripheral and the central nervous system. The ability to synthesize acetylcholine and the concentration of cholinesterase appear to diminish together with the decrease of acetylcholine in the more highly developed brains. It is natural to suggest that the transmission by acetylcholine may be a more primitive type of central transmission, the importance of which receded in higher animals, and which is retained in them at certain central synapses only. Many facts, however, cannot be explained in this way and our knowledge at present is too scanty to justify a definite statement of this kind, for instance, the sensitivity of the nerve cells may increase in higher animals.

(2) The sensory fibres contain no acetylcholine or only traces, similarly they can only synthesize it in small amounts if at all. How then are we to explain the transmission by acetylcholine of impulses from these fibres to the cells in the spinal cord? We should have to assume as pointed out by Loewi and Hellauer (1938), that the sensory fibres at their endings acquire new properties, an assumption for which there is at present no experimental evidence.

(3) Whereas the effects of acetylcholine and of eserine on the motor cortex and in the spinal cord, as well as the release of acetylcholine from the cord during increased reflex activity, suggest synaptic transmission by acetylcholine in the central motor pathway, the fibres of the pyramidal tract contain extremely small amounts of acetylcholine. This makes it difficult to consider them as cholinergic fibres.

(4) The cerebellum is rich in synapses but again contains only traces of acetylcholine and its ability to synthesize acetylcholine is small. Against this fact must be placed the one experiment of the effectiveness of eserine in a large concentration on local application to the cerebellar cortex.

If we must assume that acetylcholine is not the universal central transmitter then our conception concerning its rôle in the central nervous system will be influenced greatly by the view we take concerning the transmission across those synapses not effected by acetylcholine. Must we assume different transmitter substances in the central nervous system or must we assume that, in some, excitation is effected by the circulating currents from the presynaptic terminations? In that case both transmission processes might be involved in the transmission across a single synapse, the relative importance of the one or the other varying at different synapses. Bronk (1939) argues for such a pluralistic theory even with regard to transmission across sympathetic ganglia. It is fruitless at present to try to force the issue, as we do not know if nerve cells can be activated solely by the circulating currents in their sy

Lorente de Nó (1935) found that a subliminal afferent volley and a subliminal induction shock applied apparently directly to a central motor neuron can sum to produce threshold excitation. From this observation he concludes that cell body and dendrites with their synaptic surfaces are electrically excitable and that therefore the action current is the central synaptic transmitter. There are, however, experiments which suggest that the cells of sympathetic ganglia, at least, cannot be excited in this way. We have mentioned the inability of the nerve impulse to cross the sympathetic synapse on reaching the ganglion in the early stage of nerve degeneration when acetylcholine has nearly disappeared from the nerve and its ability to synthesize acetylcholine has been lost. Further proof is found in the regeneration experiments of Langley and Anderson (1904). As Dale has pointed out, their results show that only cholinergic fibres, and not sensory or adrenergic fibres, are able to make functional connection with a sympathetic ganglion. If it had been shown that the regenerating adrenergic or sensory fibres had reached the ganglion and had conducted impulses right up to the cells without however exciting them, these experiments would have furnished strong proof that the electrical variation of the nerve impulse itself is insufficient for excitation.

Similar experiments carried out with skeletal muscles have given conflicting results. In mammals the results were the same as in the ganglion, that only cholinergic fibres were able to enter into functional junction with the motor endplates (Langley and Anderson, 1904). On toads, however, Weiss (1934, 1935) obtained different results. When the central end of a sensory root was allowed to grow into a denervated striped muscle electrical stimulation of the sensory nerve produced muscular contractions in a few experiments, but the impulses set up in these nerves by natural stimulation from the skin never had this effect. It is thus doubtful that real sensory fibres had made functional contact with the muscle. However when the peripheral end of the sensory nerve was allowed to grow into the denervated muscle it always contracted on electrical stimulation of the regenerated sensory nerve. As sensory nerves may contain a few cholinergic fibres we do not know if the circulating current of the impulse itself had the excitatory effect. The acetylcholine content of the regenerating fibres has not been determined. A further difficulty in assessing the bearing of the experiments of Weiss on the problem of the excitability of motor endplates lies in the fact that the regenerating nerve fibres ended on the muscle fibre and not on specified plates. Experiments on endplates have been performed by Buchthal and Lindhard (1939) in muscles of lizards. According to their results we must assume two boundary faces with different properties of excitability, a boundary between the nerve ending proper and the sole, and a boundary between the sole and the muscle fibre. The latter is certainly excitable electrically but the former may possibly be excitable to acetylcholine only. At least when it is blocked by excess of acetylcholine to the action of acetylcholine, it is also unaffected by the nerve impulse. The electrical inexcitability of denervated plates of electric organs of fishes also suggests electric inexcitability of motor endplates at the boundary facing the nerve terminations.

The main danger at present for the theory of central transmission by acetyl-

choline is not the difficulties which oppose it, but the attempt to base the theory on single items of evidence. It is possible that a completely new approach must be found in order to settle the problem. One new approach might be provided by injecting large amounts of purified cholinesterase into animals or organs and to study the effect on the transmission process. Mendel and Hawkins (1943) have applied this method to the light reflex but the effect of the cholinesterase in their experiments was, as they themselves consider, mainly on the acetylcholine released from the parasympathetic endings in the pupil.

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## GENETICS AND METABOLISM IN NEUROSPORA

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Within recent years there has been a noticeable shift in emphasis in the field of genetics away from studies on the mechanism of gene transmission to investigations in such subfields as population genetics, the physical and chemical nature of genes, the mechanism of gene mutation, and the manner of gene action in development and function. The last of these topics is often referred to as physiological or biochemical genetics. It is this branch of genetics as it is approached through studies on the fungus *Neurospora* with which the present review is primarily concerned.

There are a number of instances known in which the influence of specific genes can be referred to more or less well known chemical processes. Among these are gene-antigen relations, flower pigmentation, specific abnormalities in phenyl alanine-tyrosine metabolism in man, mating reactions in protista, pigmentation in animals, sugar fermentation by yeasts, atropine esterase activity in rabbits, and a number of others [see reviews by Wright (66, 67), Lawrence and Price (30), Haldane (22, 23), Sonneborn (50), Ephrussi (16), and Beadle (3) for details and references]. Consideration of examples such as these has led to the suggestion that there exists for most genes a one-to-one relation between gene and specific reaction (5, 27). This implies that the primary action of a gene is in the control of a specific reaction.

With this concept in mind the general problem has been approached in *Neurospora* in a manner somewhat different from that usually followed (5). If it is true that for every biochemical reaction, or perhaps more precisely every enzymatically catalyzed reaction, there is in primary control one specific gene, it should be possible to select a reaction which it is desired to investigate and by means of induced mutation discover the gene in primary control of it. Some categories of reactions are obviously simpler to work with than others and a deliberate selection has been made of those in which the products can be supplied from outside the organism. Reactions leading to the synthesis of amino acids and vitamins, for example, fall into this classification. An organism that cannot synthesize thiamin can survive if this vitamin is supplied to it from an external source. Therefore, if a sufficiently large number of gene mutations were induced in an organism which could synthesize this substance, some of them might be expected to result in a loss of the ability to make this specific compound. In this way it should be possible to convert an organism originally independent of an external source of thiamin into one dependent for its existence on such a supply. Experimental control can be had by regulating the amount supplied of this vitamin which we are already fairly certain is necessary for all organisms.

The study of such reactions necessitates the choice of an organism in which the

raw materials supplied are completely under the control of the investigator. This limitation immediately excludes the use of all higher plants and animals since these cannot readily be grown on chemically defined media and under aseptic conditions. It suggests the use of micro-organisms. But all bacteria and blue-green algae are out of the question because, without sexual reproduction, genetic control in the usual sense cannot be had. Certain green algae suggest themselves, but breeding technics in these are usually not simple. This is likewise true of protozoa, and they suffer from the additional disadvantage of having complex and not completely understood dietary requirements. Fungi, on the other hand, seemed more promising. The ascomycete *Neurospora* has a life cycle almost ideally suited to genetic studies, can be grown under aseptic conditions, is not unreasonable in its space requirements, and has been studied rather extensively from a genetic standpoint (10-14, 32, 49). Its one disadvantage at the time it was first considered was that its growth factor requirements were not known. Investigation soon showed, however, that all commonly cultured species can be grown on a medium containing inorganic salts, an inorganic nitrogen source, any of a number of carbon sources such as glucose, sucrose, starch or fat, and the growth factor, biotin (5, 7). At the time of these studies the chemical nature of biotin was not known but it was nevertheless available for experimental purposes either as a concentrate or as pure crystalline material. Fortunately, its structure is now known and synthetic material has become available.

I THE ORGANISM A *Historical Neurospora*, known in its imperfect or non-sexual stages as *Monilia*, was called to the attention of science over one hundred years ago as a pest in bakeries. It grows prolifically on bread, producing masses of salmon colored conidia which are dispersed in dense clouds when disturbed. Before the days of mold inhibitors in commercial bread, a bakery infested with *Neurospora* was in for serious difficulties. On this account it was the subject of several scientific inquiries in the last century. (See Shear and Dodge, 49, for accounts of these.) With the discovery of the sexual stage by Shear and Dodge and with the finding that the ascospores can tolerate remarkably high temperatures (see below) it became clear that a possible reason for the difficulty of controlling the mold lies in the fact that the sexual spores are able to withstand the temperature attained in the interior of loaves of baking bread.

The genus *Neurospora* is widely distributed in the tropics where it frequently is a first colonizer of burned-over areas. Vorderman (62a) states that in the East Indies, following burning in connection with volcanic eruptions, *Neurospora* completely overgrows the charred remains of vegetation, producing great masses of brilliant conidia, and creating a most bizarre appearance. The resistance of its sexual spores to heat of course gives it a great selective advantage under these conditions.

Near the close of the last century the well-known Dutch botanist, F. A. F. C. Went, was stationed in Java. He was interested in fungi used for technical purposes and his attention was called to *Monilia sitophila*, now known as *Neurospora sitophila*, which was used by the Javanese in making a kind of delicacy

known as "ontjom" According to Heyne (23a), Vorderman (62a) and Went (64) ontjom are prepared from peanut meal from which the oil has been expressed. Layers of this residue are spread on banana leaves and seeded with *Neurospora* spores. After several days' growth of the fungus, during which the peanut meal is fermented and brilliant orange conidia are produced in profusion, the ontjom are ready for consumption.

Prof F. A. F. C. Went undertook to study the "ontjom fungus" from a physiological standpoint. He encountered serious difficulty, however. At the high humidities of the tropics the fungus has the discouraging habit of growing rapidly through the cotton stoppers of the containers in which it is cultured. The investigations were deferred until his return to Holland. There '*Monilia sitophila*' could be cultured readily. He described it in detail (64) and carried out various physiological studies in which he investigated its growth in chemically defined media containing various carbon and nitrogen sources and in which he made a remarkably thorough investigation of the ability of the organism to produce enzymes extracellularly and in response to specific substrates (63, 64, 65).

In all of Went's investigations biotin was supplied inadvertently as an impurity and from the data on dry weights of mycelia produced was evidently a limiting factor in most instances. In a discussion of the results of experiments in which raffinose was added to media containing other sugars, Went came close to discovering that some accessory factor (biotin) was necessary for the growth of *Neurospora*. He presents an elaborate discussion of the disproportionate effect of small amounts of raffinose, and as one reads this, it is difficult to see why the idea of an impurity in the raffinose did not occur to him. It is, of course, easy to see the explanation now, but one must remember that Went wrote in 1901, about the time Wildiers suggested accessory growth factors for yeast and many years before the concept of vitamins, as we now know it, took definite form.

The year 1927 marks the birth of the genus *Neurospora* (literally "nerved spores") and the beginning of its use in genetic studies. In this year Shear and Dodge (49) published their account of the life histories of members of the genus, including the sexual stages. They proposed the generic name and described the heterothallic species, *sitophila* and *crassa*, and the homothallic species, *tetra sperma*. Dodge immediately recognized the advantages of *Neurospora* for genetic studies and undertook a series of investigations which have been continued without interruption since (10-14). He determined the manner of sex inheritance, demonstrating that segregation for the gene pair concerned might occur at either the first or second meiotic division in *N. sitophila*. Albinism (more precisely, failure to form conidia) in *N. sitophila* was shown to be inherited in a simple fashion with genetic segregation occurring in either the first or second meiotic divisions.

With characteristic enthusiasm Dr. Dodge pointed out to Prof. T. H. Morgan, then at Columbia University, the advantages of the new material for genetic studies. Morgan carried a collection of cultures to the California Institute of Technology in 1928 where he maintained them for a period of about a year.

They were eventually turned over to Dr C C Lindegren who has since made substantial contributions to our knowledge of the genetics of the organism

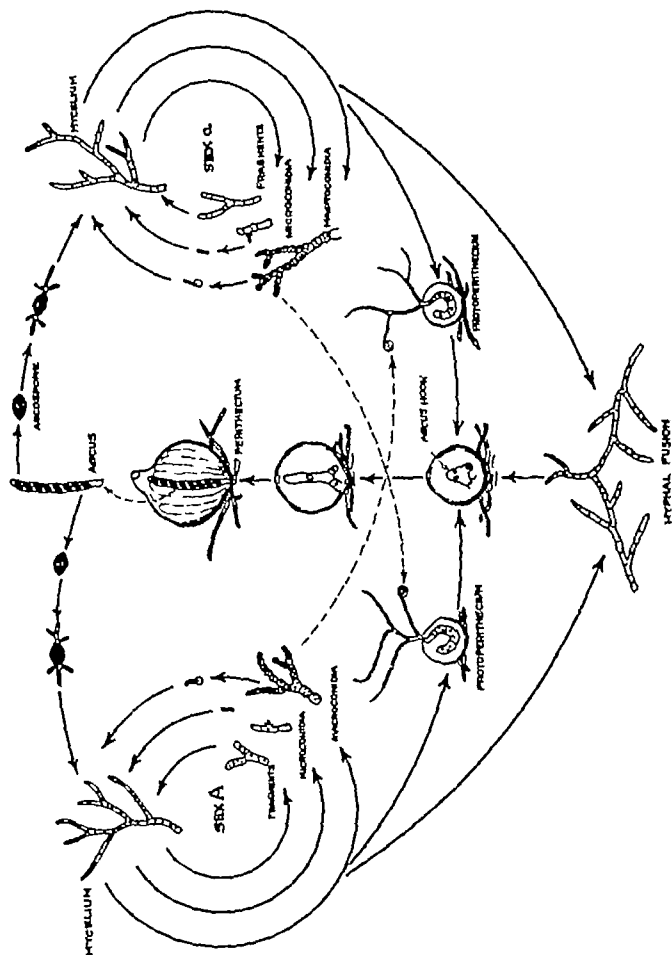
**B Life cycle.** An understanding of how *Neurospora* is used experimentally in biochemical genetic studies necessitates an appreciation of certain features of its life cycle. These are shown diagrammatically in figure 1 for heterothallic species which are most convenient for genetic studies. The vegetative hyphae are cylindrical tubes with septae at intervals. Each segment is multinucleate with perhaps as many as twenty nuclei. Each septum has a central perforation large enough to allow passage of hyphal nuclei. Thus the mycelium is essentially coenocytic. The individual nuclei are haploid, carrying a single set of seven chromosomes (McClintock, personal communication, 38)

On a suitable medium, mycelia grow rapidly by a process of elongation of individual hyphae at their tips and through profuse hyphal branching. Some few days after growing over a suitable medium, chains of asexual spores are cut off at the tips of aerial hyphae (conidiophores). Most of these conidia contain several nuclei although an occasional one may be uninucleate. A second type of asexual spore known as a microconidium is also produced (13). According to Lindegren and Lindegren (33, 34) microconidia are uninucleate. Vegetative reproduction therefore occurs in three ways: 1, by means of conidia, 2, through microconidia, or 3, by way of hyphal or mycelial fragments.

*Neurospora crassa* and *N. sitophila* are heterothallic, i.e., strains of two sexes exist. These are morphologically identical so far as we know but are physiologically distinct as shown by their mating reactions. These two sexes are usually kept in separate cultures in the laboratory. They are designated *A* and *a* types (or *A* and *B* according to earlier nomenclature of Dodge, or + and - in Lindegren's terminology).

If spores or hyphae of the two sexes are placed together on a suitable medium, fusion of hyphae occurs and sexual fruiting bodies known as perithecia are initiated. Within these perithecia, fusion between nuclei of opposite sexes occurs in structures known as ascus hooks. The resulting diploid zygote nucleus undergoes two meiotic divisions the details of which follow the conventional pattern (10, 38). These occur in such a way as to give four haploid nuclei lying along the axis of the spore sac or ascus. There follows a mitotic division of each of the four primary meiotic products, giving a row of eight nuclei each with seven chromosomes. Each is included in a sexual spore (ascospore) which is an ellipsoidal, black body about 28  $\mu$  long with longitudinal ridges on its surface (49). A further mitotic division follows by which the ascospores become binucleate, their condition at maturity.

On cornmeal agar or other suitable media, both *A* and *a* strains of *N. crassa* or *N. sitophila* produce "protoperithecia" (13, fig. 1). These are spherical masses of hyphae from which specialized hyphae known as trichogynes protrude. In the absence of hyphae or spores of the opposite sex the proteperithecia remain as infertile bodies. If conidia or microconidia of the opposite sex come in contact with the trichogynes, connecting pores develop by which the contents of the conidia enter the trichogyne (1). The nuclei of the conidia migrate





through the trichogyne and fuse with nuclei of the opposite sex in the ascogonium. The protoperithecium then develops further and becomes a fertile perithecium. "Fertilization" of protoperithecia may be brought about by conidia, microconidia, or hyphae. Crosses between strains can usually be made by using either sex as the protoperithecial parent. It should be noted, however, that many mutant strains are poor producers of protoperithecia and in crossing two such strains it is often desirable to make the cross by the method of hyphal fusion.

Unlike *N. crassa* and *N. sitophila*, *N. tetrasperma* is "homothallic" in nature, i.e., has only one type of mycelium which is by itself capable of reproducing sexually. Dodge (10) has shown that this condition arises because of the presence within individual hyphae of nuclei of the two sexes. Heterothallic races can be obtained experimentally (14). The mechanism of ascospore formation in tetrasperma is such that, unless it fails to work precisely, each of the four spores of an ascus contains two nuclei of each sex. In other respects *N. tetrasperma* is very much like the heterothallic species. Dodge has, in fact, shown that hybrids between tetrasperma and crassa are possible and that they are at least partially fertile (10a).

**C Heat activation of ascospores** The ascospores of *Neurospora*, like those of certain other ascomycetes, require activation before they will germinate. The only way known by which this activation can be accomplished is by exposure to high temperature (60°C for 30 minutes is a standard treatment). This interesting property was discovered in *Ascobolus* by Dodge through chance observations on ascospores that had been accidentally placed in a drying oven rather than in an incubator (9, and personal communication).

The mechanism by which ascospores of *N. tetrasperma* become heat-activated has been shown by Goddard to involve specific stimulation of the carboxylase system (18, 19, 20). The same mechanism undoubtedly applies to the heterothallic species *crassa* and *sitophila* since their requirements for heat-activation appear to be the same as those of *tetrasperma*. Activation is an all-or-none process and can be reversed if activated spores are placed under anaerobic conditions. Whether heat-activation of the carboxylase system involves the enzyme protein or cocarboxylase is not known, although the high heat of activation suggests that denaturation of a protein may be concerned (19).

Lindegren (31) has reported that genetic differences exist in *N. crassa* with respect to conditions required to activate ascospores. Some spores germinate without heat treatment, but how they differ genetically from spores requiring heat-activation is not entirely clear.

**D Genetic segregation** From the arrangement of nuclei during the three divisions by which the eight primary ascospore nuclei arise (10) and from the fact that meiosis follows the usual path (38), regular segregations should be observed of alleles of genes heterozygous in the diploid fusion nucleus. This has been observed to be the case by both Dodge (11, 12, 13) and Lindgren (32). The eight ascospores of *crassa* and *sitophila* are regularly four of one sex and four of the other. Numbering ascospores from the tip of the ascus, their arrangement with regard to sex has been shown to be any one of the following

A A A A a a a  
 a a a A A A A  
 A A a A A a a  
 A A a a a A A  
 a a A A A a a  
 a a A A a A A

The first two arrangements represent first division segregations of the two alleles of the sex gene (or possibly a differential segment of a chromosome), while the remaining four types show second division segregation of the two

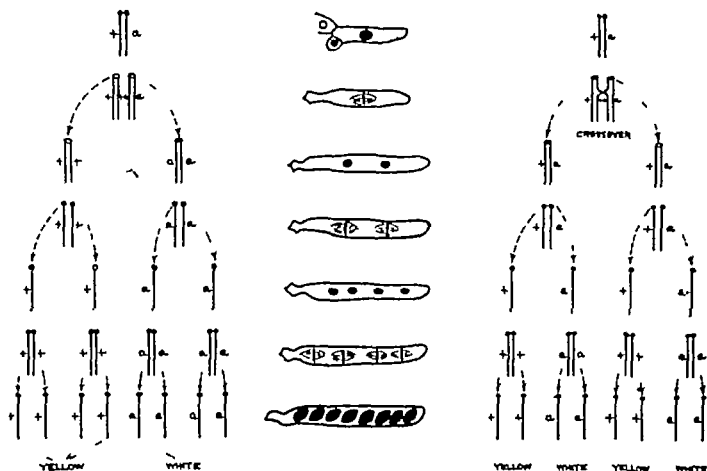


Fig 2 Nuclear and chromosome history in developing ascus from cross of wild type and albino strains. Nuclear divisions shown in center column. Left chromosome basis of first division segregation. Right chromosomal basis of second division segregation. Two alleles of segregating gene are designated + (normal allele) and a (mutant allele).

alleles. The chromosomal basis of these two types of segregation is shown in figure 2 in which segregation of yellow and albino strains in *N. crassa* is represented.

The occurrence of a chiasma between the centromere and a segregating gene pair results from physical exchange of corresponding segments of homologous chromosomes and is expressed in the final arrangement of spores as second division segregation. The frequency of formation of such chiasmata between the centromere and a given gene locus is dependent on the distance of the gene from the centromere. Hence frequency of second division segregation is a

measure of distance of the gene pair concerned from the centromere. This distance can be determined in terms of the same cross-over or map units by which gene arrangements are expressed in higher plants and animals.

From a genetic standpoint, an organism like *Neurospora* has several advantages. In the first place, the haploid phase is the one usually studied. There are therefore no complications due to dominance, and the ratios of types measure directly the proportions in which meiotic products occur. Secondly, since all products of meiosis are recovered, basic ratios are mechanically determined and are therefore not subject to sampling errors. Finally, strains can be multiplied rapidly by vegetative means and it is thereby possible to obtain any desired amount of genetically homogenous material.

**E Heterocaryosis** Dodge (14) has shown that between two strains (artificially produced) of *N. tetrasperma* of the same sex but differing genetically in other characters, hyphal fusions occur giving rise to mycelia with two genetic types of nuclei, each haploid. Beadle and Coonradt (4) have shown that such vegetative fusions of hyphae of the same sex occur regularly in *N. crassa*. The resulting heterocaryotic strains are useful in genetic studies in determining whether a mutant gene allele is recessive or dominant to the normal allele from which it arose. They are likewise of value in determining whether two mutant alleles of independent origins are alleles of each other or represent mutant changes in separate genes. Physiologically, heterocaryons are the equivalent of heterozygous diploids if they involve two component genetic strains.

**F Methods of culture** The media on which *Neurospora* strains are cultured vary depending on the nature of the strain and how the culture is to be used. For ordinary cultures any standard fungus medium is satisfactory. For crosses in which ascospore production is desired, Difco cornmeal agar is often used. In biochemical experiments a basal or "minimal" medium is used having the following composition in grams per liter: ammonium tartrate, 5; ammonium nitrate, 1; monobasic potassium phosphate, 1; magnesium sulfate ( $7 \text{ H}_2\text{O}$ ), 0.5; sodium chloride, 0.1; calcium chloride, 0.1; sucrose, 20; biotin,  $5 \times 10^{-6}$ . In addition, it contains the following trace elements, added as salts, in milligrams per liter: B, 0.01; Mo, 0.02; Fe, 0.2; Cu, 0.1; Mn, 0.02; Zn, 2.0. This medium may be used in liquid form or made semisolid with 1.5 per cent agar. If agar is added, care should be taken that materials undesirable in a given experiment are not inadvertently added with it. Especially washed agar is often necessary (44, 45). For particular experiments the above minimal medium may be supplemented with desired compounds, or otherwise modified.

Ryan, Beadle and Tatum (45) have made a detailed study of growth on solid medium in "growth tubes". In these, progression of a mycelial frontier along a horizontal agar surface in a glass tube can be measured conveniently. A similar quantitative measure of growth on agar surfaces in petri plates has been investigated (62). Quantitative measures of growth in liquid medium can be made by weighing dried mycelial pads from Erlenmeyer culture flasks of any desired size, e.g., 125 ml flasks containing 20 ml of medium (26).

Single ascospore cultures, necessary for genetic control, are readily obtained by transferring ascospores on small blocks of 3 per cent agar to small test tube slants.

Isolations may be made either at random or in order from individual asci. Any conidia, microconidia, or bits of mycelium inadvertently transferred with an ascospore are almost invariably killed at the high temperature at which ascospores are activated (30 min. at 60°C). In order to allow ascospores to age before heat treatment and to make doubly sure that no vegetative spores or fragments contaminate single ascospore cultures, it is often desirable to flood individual ascospores with a 1.5 per cent solution of sodium hypochlorite. This is effective in killing both vegetative spores and mycelial fragments (Beadle, unpublished observations).

**II PRODUCTION OF MUTANT STRAINS DEFICIENT IN SPECIFIC SYNTHESIS**  
The theory and practice of producing and selecting mutant strains of *Neurospora* in which specific synthetic reactions are blocked have been described by Beadle and Tatum (5, see also reviews 27, 57, 58). On the assumption that all enzymatically catalyzed reactions bear a rather direct relation to specific genes, it should be possible, by producing gene mutations at random in a sufficient number of strains, to select those in which almost any synthesis is interrupted.

It is known that ionizing radiation such as x rays, gamma rays, and neutrons, produce relatively permanent changes in genes. These may represent physical destruction of genes, reversible inactivations or other changes (40). Mutations are likewise produced following absorption of ultraviolet radiation. These appear to differ at least statistically from those produced by ionizing radiation in that they involve a smaller proportion of physical losses and physical rearrangements of chromosome parts and a greater proportion of potentially reversible gene changes (51, 52). It should be recognized that valid deductions can be made as to the rôle played by a given gene whether we determine this rôle by experimentally eliminating the particular gene or by partially or completely inactivating it while leaving its power of self-duplication unimpaired.

The procedure employed in producing "biochemical mutants" in *Neurospora* (5, 27) involves raying conidia either dry (with x rays or neutrons) or suspended in liquid minimal medium (ultraviolet). X-ray dosages up to 100,000 r units may be applied to dry conidia. Ultraviolet radiation from a Westinghouse sterilamp which emits some 90 per cent of its energy at 2537 Å, has been used. This wavelength is near the maximum absorption of nucleic acid which Stadler and Uber (52) and others have shown to coincide with the maximum sensitivity of genes to ultraviolet-induced mutation. At this wavelength some conidia will tolerate 15,000 ergs per mm<sup>2</sup>. Following treatment, conidia are applied to protoperithecia of the opposite sex. From the mature perithecia so produced ascospores are taken—one ascospore per perithecium to avoid obtaining the same mutant twice. These are grown in small individual test tube cultures. Since mutations are induced prior to meiosis, the vegetative descendants of a single ascospore are genetically homogenous, barring further spontaneous mutation. This would not be so if mature ascospores or conidia were treated and their direct vegetative descendants investigated. It may not be true if the direct descendants of uninucleate microconidia are used (33, 34), since it is possible that chromosomes may be effectively double even in a haploid nucleus.

Single ascospore strains are grown on a medium made as complete as possible

in vitamins, amino acids and other growth factors so that any induced deficiency in synthetic ability will be compensated for by a component of the medium. After establishment on "complete" medium, vegetative transfers (conidia) are made of strains to a minimal medium. If normal growth occurs on various minimal media (with carbon and nitrogen sources varied and with other factors such as pH and temperature varied), the culture is considered to be like the original wild type strain and is discarded. If, however, growth fails to occur on one or more minimal media, a mutation blocking some essential reaction is assumed to have taken place. By making a systematic series of tests on minimal media supplemented in various known ways, it is possible to determine something about the nature of the altered growth requirements of a particular strain. For example, if growth occurs on a minimal medium supplemented with pyridoxin but not on media in which this vitamin is absent, it is assumed that some essential reaction in the synthesis of this compound has been interrupted.

Once mutant strains have been identified it is a simple matter to determine how they differ genetically from the original strain. Crosses with wild type of the opposite sex are made. From a number of asci the eight ascospores are taken in order and grown in individual cultures on a medium capable of supporting growth of both mutant and wild-type strains. After cultures have become established on this medium, vegetative transfers to a suitable minimal medium are made. If from each set of eight spores four strains are obtained which fail to grow on unsupplemented minimal medium and four capable of doing so, and if the normal and mutant spores are in the expected arrangements, we are justified in concluding that the mutation involved an alteration of a single point in a chromosome, i.e., was a single gene change or single gene loss. There is some possibility that simultaneous loss of several adjacent genes would give the same genetic result as loss or inactivation of a single gene but it does not seem probable that such a multiple defect could be compensated for by supplementing the minimal medium with a single chemical compound.

Some 60,000 single spore strains, the parents of which were treated with x-rays, neutrons, or ultraviolet, have been tested on various minimal media (27). It is not possible at present to give an exact figure for the percentage of biochemical mutants among these, since many have been classified tentatively only and require further study. Nor is it possible to say how many genes have been involved in mutants of this class since recurrences of the same mutation are often found. Without detailed genetic and biochemical tests these cannot be distinguished from mutations in separate genes concerned with different steps in a sequence of reactions leading to a particular end product. It is probably a fair estimate, however, that at least 100 genes affecting vital reactions have been mutated (27). The majority of these involve the synthesis of vitamins, amino acids or nucleic acid components. However, it is evident both on theoretical grounds and in experimental practice that the types of mutants detected will depend on the conditions under which the selection is made. For example, yeast extract is a constituent of the usual "complete" medium. This is known to contain substances specifically inhibitory to certain mutants and it is clear

that a medium containing it does not favor the selection of these mutants. Furthermore, it has been shown that specific mutant types grow normally on a minimal medium at one pH but not at another (53, 55). Their detection obviously depends on the use of a minimal medium of a suitable hydrogen ion concentration. After the detection of mutant strains showing defects in synthetic ability, the classification of these in terms of the reactions concerned depends on key compounds being both known and available for use. There is a strong tendency to concentrate on those mutants that are most easily classified and this of course means selection of those concerned with synthesis of compounds of recognized nutritional significance. Too much weight should not therefore be assigned to the fact that most of the mutants reported involve these compounds. Even making allowance for these biasing factors, however, most of the clear-cut mutants have been shown to involve synthesis of known compounds. From a statistical standpoint this means that there are probably not many more members of the B-complex of vitamins to be discovered. Similar reasoning indicates that the majority if not all the natural amino acids of nutritional importance are known.

III ARGININE SYNTHESIS Srb and Horowitz (55) have analyzed fifteen mutant strains, the growth requirements of which are met by arginine. Seven of these proved to be genetically distinct. The remaining eight represent recurrent mutations of genes involved in the first seven.

These arginine mutants may be used to illustrate the methods of demonstrating genetic identity or non identity of mutant alleles. If two mutant strains result from mutation of the same gene, a cross between them will yield only mutant offspring. Furthermore, a heterocaryon between them will show the same growth requirement as the mutant strains—it is the physiological equivalent of a homozygous diploid in higher plants and animals.

If, on the other hand, two strains requiring arginine or a related compound for normal growth result from mutations of different genes, the cross between them will yield both mutant strains and strains which like the original wild type grow in the absence of arginine. If the two mutant genes are closely linked, there will be few wild type offspring produced, if they are carried in separate chromosome pairs, wild type descendants will be expected to constitute 25 per cent of the total. In certain types of asci it is possible to identify double mutant spores even though physiologically these may not be distinguishable from one of the component single strains. For example, if  $a_1$  and  $a_2$  represent mutant alleles of two genetically independent genes concerned with arginine synthesis, while  $A_1$  and  $A_2$  symbolize their corresponding normal alleles, the zygote nucleus will have the constitution  $A_1/a_1 A_2/a_2$ . If both pairs segregate at the first meiotic division and the mutant alleles are carried in the same end of the ascus, the eight spores will be as follows

$$A_1A_2, A_1A_2, A_1a_2, A_1a_2, a_1A_2, a_1A_2, a_1a_2, a_1a_2$$

or the equivalent of this with the entire order reversed. The fact that four spores received normal alleles of both genes shows that each of the remaining four

spores must carry the two mutant alleles. The same reasoning would of course apply if both gene pairs showed second division segregation and four spores grew normally on minimal medium. The above genetic diagnosis of double mutant spores can be confirmed by crossing strains derived from them to wild type. If the identification is correct, some of the asci from the cross will carry eight mutant spores, a result not possible if the mutant strain differed from wild type by a single gene. The genetic nonidentity of the two mutant alleles  $a_1$  and  $a_2$  can be confirmed by the heterocaryon test. If  $a_1$  and  $a_2$  strains of the same sex are grown together on a medium containing a small amount of arginine (the amount carried over in conidia is sufficient) hyphal fusion will occur giving rise to a mycelium containing two types of nuclei  $a_1A_2$  and  $A_1a_2$ . If both  $a_1$  and  $a_2$  are recessive to their normal alleles the heterocaryon will be the physiological equivalent of a double heterozygote in a diploid and will grow in the absence of arginine. Because of the possibility of dominance of either or both mutant alleles, however, a failure of the heterocaryon to grow normally cannot be accepted as evidence of allelism of  $a_1$  and  $a_2$  (4).

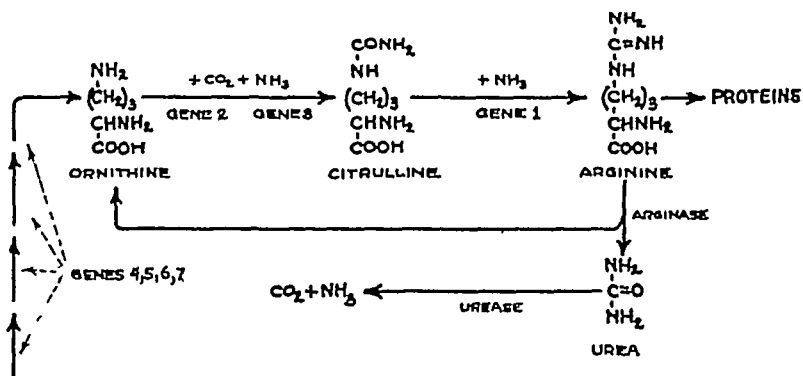


Fig 3 Ornithine cycle in *Neurospora*

In the analysis of "arginineless" mutants, Srb and Horowitz made use of these types of genetic tests. Characterization of the seven genetically distinct mutants in terms of their requirements for normal growth showed that one responded only to arginine, two grew normally in the presence of either arginine or citrulline, while the remaining four responded to arginine, citrulline, or ornithine. It is significant that for the conversion of citrulline to arginine which is most probably a single reaction, only one gene is known. Two genes are known for the conversion of ornithine to citrulline and there are good chemical reasons for supposing that this transformation involves two reactions with carbamino ornithine as an intermediate. The reactions by which plants synthesize ornithine from simpler constituents are not known. Four genes have been shown to be concerned with reactions leading to ornithine and on the basis of the one-gene-one-reaction concept this would imply at least four reactions in its synthesis, an implication that is certainly not unreasonable from a chemical standpoint.

In *Neurospora*, as in the mammalian liver, arginase is present and catalyzes the splitting of arginine into ornithine and urea. Unlike the animal which

excretes the urea, *Neurospora* further degrades it into carbon dioxide and ammonia under the influence of the enzyme urease. It is clear that the only reasonable interpretation of the combined genetic and biochemical data involves the assumption that in *Neurospora* there is an "ornithine cycle" (fig 3) fundamentally similar to that postulated for the mammal by Krebs and Henseleit (29). The part played by genetics in the discovery of this in *Neurospora* is evident. Without the mutant strains studied by Srb and Horowitz it would have been difficult indeed to demonstrate. From the standpoint of comparative biochemistry, it is most significant that organisms as far apart as *Neurospora* and mammals should have systems of reactions involving arginine that are so strikingly similar. It is probable that the ornithine cycle constitutes a basic process in the metabolism of protoplasm in all organisms.

**IV BIOSYNTHESIS OF TRYPTOPHANE** A frequent type of mutant in *Neurospora* resulting from x ray or ultraviolet treatment is one requiring an exogenous

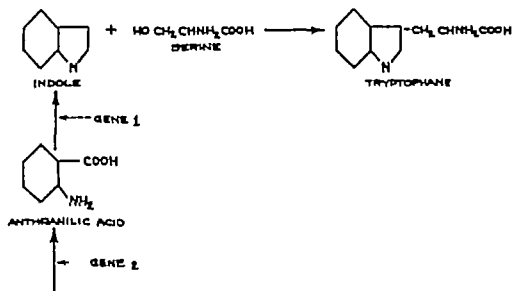


Fig 4 Tryptophan synthesis in *Neurospora*

source of tryptophane or some related compound for normal growth. Among more than 30 occurrences of mutants of this category genetic tests show at least two to be distinct. Crosses between them gave wild type recombinations and a heterocaryon resulting from their union shows substantially normal growth in the absence of tryptophane (61). Tatum and Bonner (60) have shown that for one of these mutants indole can replace tryptophane. In studying the uptake and utilization of indole, it was noted that the presence of serine in the medium markedly accelerates the disappearance of indole. The further observation was then made that following the uptake of serine and indole, tryptophane appears in the medium. The hypothesis that this is formed by condensation of indole and serine is supported by several lines of evidence (60). As a matter of fact, wild type strains are as capable of carrying out this reaction as the mutant strains used in the first experiments, but it is interesting to reflect that the experiments would in all probability not have been undertaken in the first instance had it not been for the mutant strain.



A second type of mutant can utilize either indole or anthranilic acid in place of tryptophane, while anthranilic acid cannot be used by the first one. This suggests anthranilic acid as a precursor of indole and this suggestion gains strong support from the finding that in the presence of a minimal amount of tryptophane or indole (for growth), the first mutant strain accumulates anthranilic acid and releases it into the medium. It has been isolated in pure form and identified chemically. Evidently the mutant gene of strain 1 is related to the reaction by which anthranilic acid is converted to indole. In mutant 2 the synthesis of anthranilic acid is blocked. These relations are shown diagrammatically in figure 4. It is interesting that no mutant has so far been found in which the condensation of indole and serine is blocked. There has been found a mutant type in which serine synthesis is interfered with in some way (Hungate, unpublished, 27) but detailed studies on this have not yet been reported.

These studies on tryptophaneless mutants are an example of how genetics can be of use in studying metabolic processes. It is almost exactly analogous to the classical experiments on alcaptonuria in man in which 2,5-dihydroxyphenylacetic acid is accumulated because of a genetic block preventing its further oxidation (17). This intermediate in the breakdown of phenylalanine and tyrosine would probably not have been discovered had it not been for alcaptonurics. In normal individuals it is apparently a transitory intermediate in metabolism and seldom if ever accumulates in sufficient amount to permit of its identification. Similarly in tryptophane metabolism, anthranilic acid is normally transitory and would not be easily identified without using genetic blocks to "isolate" it as a metabolic step.

V DEFECTS IN THE SYNTHESIS OF OTHER AMINO ACIDS. In addition to mutants concerned with arginine and tryptophane synthesis, strains have been detected in which lysine, leucine, isoleucine-valine, valine, methionine, and proline are required for normal growth (27). Not all of these have been subjected to detailed study, however.

Doermann (15) has described a strain requiring an external source of lysine for normal growth. It is differentiated from the normal strain by a single gene which lies on the sex chromosome. An interesting property of this strain is that it is specifically inhibited by arginine. If the molar ratio of arginine to lysine exceeds one, regardless of the absolute amounts of either in the medium, growth is inhibited. This inhibition is removed by adding an excess of lysine regardless of the absolute amount of arginine present. No inhibiting effect of arginine on wild type strains has been observed at any concentration. The explanation of this phenomenon of specific inhibition is not obvious. It is possible, as Doermann suggests, that it may involve some mechanism in which competition plays a part.

Regnery (43) has studied a mutant strain, differing from wild type by a single gene change, which requires leucine for normal growth. Its growth responses to various possible leucine precursors have not, however, clearly indicated the mechanism by which this amino acid is normally synthesized in *Neurospora*.

One single-gene mutant strain has been found with a double growth factor

requirement (6) This strain requires a supplement of both isoleucine and valine in a ratio approximately 7:3. All attempts to find a single compound capable of restoring its growth rate to normal have been unsuccessful. At first sight it might appear that this case does not agree with the assumption that a single reaction should be modified by a change in a single gene. It is possible, however, to suppose that in the formation of these structurally similar amino acids, there is a common reaction. There might, for example, be a common step in the formation of their carbon skeletons, each of which has a branch at the beta position. Or it is conceivable that the amination of two homologous alpha keto, beta methyl acids might be catalyzed by a common enzyme. However, attempts to determine the nature of this common step, if there be one and the structure of the natural precursors of valine and isoleucine have so far been unsuccessful, the final interpretation of the isoleucine-valine mutant must therefore await additional information.

**VI BIOSYNTHESIS OF VITAMINS** It is of interest to note that no clear case has yet been reported of a plant requiring any vitamin other than those of the B-group (47). In the hundred or more induced mutant strains in *Neurospora*, it is likewise true that none requires vitamin C or any of the fat soluble vitamins. It is possible that through some unknown systematic factor in the experimental procedure these have occurred but were discarded. This seems unlikely however. The important question arises as to the rôle, if any, of the fat-soluble vitamins in plants. If they play no part in the plant's metabolic economy, why were the reactions leading to their synthesis retained during the long course of evolution? It seems more probable that they are of basic importance in ways not yet understood than that they are elaborated purely incidentally. It is most probable, for example, that the carotenoids of higher plants are of some unknown significance for, otherwise, why should they be invariably associated with chlorophyll (56)?

So far as the vitamins of the B-group are concerned, *Neurospora* mutant strains have been found each of which requires the following: thiamin (57, 58), pyridoxin (5, 53, 57), *p*-aminobenzoic acid, (59), pantothenic acid (27, 57), inositol (2), nicotinic acid (27, 57, 58), and choline (26). Omitting biotin which is required by all strains, this list includes all known B vitamins except riboflavin and folic acid. No fungus requiring riboflavin has been reported although many if not all synthesize it (57). Recently a mutant strain of *Neurospora* has been found in which riboflavin is required, but only under special circumstances (Mitchell and Houlahan, unpublished).

A mutant strain of *N. sitophila* requiring thiamin or thiazole is known to differ from the normal strain by a single gene (5). A second strain requiring thiamin is known in *N. crassa* and here the specific error in synthesis appears to lie in the reaction by which the thiazole and pyrimidine halves of the molecule are joined since the thiazole and pyrimidine halves supplied simultaneously do not promote growth (58). A pantothenic acid requiring mutant strain of *N. crassa* appears to be unable to combine the pantoyl lactone and beta alanine components of the pantothenic acid molecule although this can be accomplished by certain other

organisms (57) If the moities are supplied separately or together as a mixture they are ineffective in promoting growth (57) An inositolless strain of *N crassa* is known No other compound is known which will promote its growth Two genetically distinct nicotinicless strains are known (4), but studies on their biochemical characteristics have not yet been completed

A strain of *N stophila* requiring pyridoxin has been reported (5) and studied in more detail by Stokes, Foster and Woodward (53) This is of particular interest in that at a pH of above 5.8 and in the presence of ammonia nitrogen, pyridoxin is synthesized in amounts sufficient to give approximately normal growth Other cases are known in which specific syntheses are dependent on pH of the medium and on other cultural conditions (27, 57), one of them in arginine synthesis in *Neurospora* (55) The mechanism by which pyridoxin synthesis is dependent on pH is not known, but two possible interpretations have been suggested (57) (1) that the limiting reaction is not completely blocked and at the higher pH has a rate sufficient to cover the needs of the organism, and (2) that normally two pathways are open for certain steps in synthesis, one taken at a low pH range, the other at a higher pH, and that only the low pH route is blocked in the pyridoxinless strain Another possibility is that a pH-sensitive threshold level of pyridoxin is needed and with the same absolute amount of pyridoxin this is exceeded at one pH but not at the other In any case, there is no need whatever to assume a change in the gene with change of media

That *p*-aminobenzoic acid is essential for the metabolism of *Neurospora* is shown by the occurrence of a mutant strain unable to grow normally in its absence (59) This strain, which differs from normal by a single gene, is able to use certain analogues of *p*-aminobenzoic acid, such as *p*-nitrobenzoic acid, but the activities of these are so low that they are probably not normal precursors of *p*-aminobenzoic acid The introduction of nitrogen into this vitamin probably precedes ring closure in its biosynthesis

Horowitz (27) has studied the interrelations of two genetically distinct strains both of which require choline or some related compound for normal growth One of these is evidently blocked between a particular precursor of choline and choline itself, while the other is blocked prior to the precursor This is judged to be so because in the presence of minimal amounts of choline the first mutant accumulates a substance which is not choline but which will replace choline in promoting growth of the second mutant (27) Studies on the nature of the precursor have not yet been completed

VII MISCELLANEOUS SYNTHESES Loring and Pierce (35) have studied a monogenic mutant strain of *N crassa* which is unable to grow normally unless supplied with a pyrimidine or a pyrimidine derivative Uracil as a free base will promote growth but has only 1/60 to 1/10 the activity of its nucleoside Cytosine is itself inactive but cytidine and cytidylic acid are as active in promoting growth as their uracil analogues These results suggest that cytosine is not a normal precursor of either its own nucleoside or nucleotide, or of uracil (35)

The first step in the utilization of nitrate by a plant apparently involves its reduction to nitrite This is then further reduced and may subsequently be

incorporated in various organic compounds. A strain differing from wild type by a single gene is known in which the reduction of nitrate to nitrite is blocked. Nitrite, ammonium, or amino nitrogen permits normal growth of this strain (27).

Horowitz (27) has studied two mutants in which fat metabolism is altered in some way. Wild type strains of *Neurospora* are able to utilize fats and certain fatty acids as sources of carbon and energy. One of the mutant strains is entirely unable to utilize any of a number of fatty acids tested, while the other is able to utilize saturated fatty acids but not their unsaturated analogues.

In addition to the types of mutants already considered, a second category is found. This includes those which grow on complete medium (containing yeast and malt extracts) and not on minimal, but which so far have not been induced to grow in the presence of any known compound. These mutants in which unknown syntheses are blocked are fundamentally no different from those already mentioned. On further study some of them will undoubtedly prove to require known substances that have not yet been tested or have not been tested under the proper conditions. There may be a final residue which requires compounds not at present suspected of having biological significance.

**VIII. MUTANT STRAINS AND BIOASSAYS.** A strain in which a particular reaction is genetically blocked is, in general, able to use any compound in the reaction chain that normally arises subsequent to the block—if the compound is able to enter the cell from the outside. If the ultimate reaction in the formation of a given end product is blocked, the strain will respond specifically to the final compound. Thus the arginineless mutant which is unable to convert citrulline to arginine responds specifically to arginine (55). On the other hand, a strain unable to synthesize ornithine will be expected to respond to arginine, citrulline, ornithine, and possibly to precursors of ornithine.

The quantitative growth response of a mutant strain to compounds active in promoting its growth often provides a basis for a useful method of bioassaying for these compounds. As is well known, such biological assays often have high sensitivity and specificity and can be used on complex mixtures. It is obvious that the specificity will vary depending on the genetic constitution. Thus, in the arginine series, any of three or more specificities are possible. If one were interested in arginine alone, the strain responding only to arginine would be selected. Usually with naturally occurring organisms the choice as to specificity is limited and this is often true with artificially produced mutant strains as well. No strain of *Neurospora* has been produced, for example, that specifically requires tryptophane, all are sensitive to indole as well.

Bioassay methods using *Neurospora* mutant strains have been developed for *p*-aminobenzoic acid (62), pyridoxin (54), choline (24, 26, 30), leucine (43, 46), and inositol (2).

**IX. MECHANISM OF GENE ACTION.** The types of mutant strains so far found in *Neurospora* probably make up a reasonable sample in indicating the kinds of reactions subject to genetic control. If to these are added those reactions known to be gene controlled in other organisms (3), there can be no slightest doubt but that the chemical versatility of the gene is great. In fact a convincing argument can be made that if suitable experimental conditions were possible and if one

looked sufficiently long, mutation could be detected in a gene concerned with any predetermined enzymatically catalyzed reaction. This has almost been achieved for certain sets of reactions in *Neurospora* and the possibilities have by no means been exhausted.

Assuming that it is true that every biochemical reaction has a specific gene directing its course, how is this control achieved? Unfortunately, we can only speculate as to the final answer to this question. Such speculation is not entirely without basis, however, and is perhaps justified by the importance of the question.

Genes are thought from various lines of evidence (3, 21, 39, 41, 48) to be composed of nucleoproteins or at least to contain nucleoproteins as essential components. They have the ability to duplicate themselves which of course they do once every cell division. The manner in which this self-duplication is brought about is one of biology's unsolved problems, but it is thought to involve a kind of model-copy mechanism by which the gene directs the putting together of the component parts of daughter genes (8, 22, 23, 66). If this is the mechanism and genes contain protein components, gene reproduction is a special case of protein synthesis. Since many cases are known in which the specificities of antigens and enzymes appear to bear a direct relation to gene specificities (3, 66), it seems reasonable to suppose that the gene's primary and possibly sole function is in directing the final configurations of protein molecules.

Assuming that each specific protein of the organism has its unique configuration copied from that of a gene, it should follow that every enzyme whose specificity depends on a protein should be subject to modification or inactivation through gene mutation. This would, of course, mean that the reaction normally catalyzed by the enzyme in question would either have its rate or products modified or be blocked entirely.

Such a view does not mean that genes directly "make" proteins. Regardless of precisely how proteins are synthesized, and from what component parts, these parts must themselves be synthesized by reactions which are enzymatically catalyzed and which in turn depend on the functioning of many genes. Thus in the synthesis of a single protein molecule, probably at least several hundred different genes contribute. But the final molecule corresponds to only one of them and this is the gene we visualize as being in primary control.

It is by no means an easy matter in all cases of gene change to determine what particular chemical reaction is modified—or even that only a single one is modified. Even after a given reaction is specified as the one modified in a particular mutant strain, the mutation may effect it only indirectly. In the case of the mutant strain of *Neurospora* that cannot combine the two components of the pantothenic acid molecule, we infer that a specific enzyme concerned with this reaction is inactivated. But it is possible, if the enzyme concerned depends for its activity on a prosthetic group or coenzyme, that the enzyme is inactive either because of an inactive protein configuration (primary gene control) or because of absence of coenzyme or prosthetic group (secondary control). Since in this particular case the enzyme concerned is not known, the distinction is not at present easily made on the basis of experimental data.

**X. EVOLUTIONARY IMPLICATIONS** From the work on *Neurospora* the genetic basis of the evolutionary specialization of organisms with regard to nutritional requirements becomes clear. Starting with a completely autotrophic organism it is easy to see how by gene mutation it could become progressively simpler so far as its synthetic abilities are concerned and correspondingly more exacting in its nutritional requirements. That just this kind of specialization has occurred in many groups of organisms is clear from the work of Lwoff (37), Knight (28), Schopfer (47) and others. With the evolutionary development of parasitism, for example, it is to be expected that, since many compounds can be obtained from the host, the ability of the parasite to synthesize these will in many instances be of no selective advantage. Since loss by mutations are known to be more frequent than the reverse it is statistically inevitable that eventually a gene that confers no selective advantage will become inactive or be lost. Among the fungi, however, Schopfer (47) points out that the ability of parasitic forms to synthesize vitamins of the B group is sometimes retained in spite of the parasitic habit. Presumably the ability of the organism to carry out these syntheses is of some advantage that is not immediately evident.

From a genetic standpoint specialization through loss of synthetic ability would be expected to be difficult but not impossible to reverse. Consider, for example the case of thiamin synthesis. If an organism able to synthesize this vitamin and incorporate it into its enzyme system is able to obtain free thiamin in abundance in the medium in which it grows, a mutation resulting in the loss of ability to synthesize it will not be selected against and will be expected largely to replace the original form as a result of mutation pressure (greater rate of mutation from the active form of the gene to an inactive form than the reverse). If the ability to combine thiazole and pyrimidine is lost in this way it is of no advantage to the organism to synthesize these components unless they serve some independent need that is not satisfied by the exogenous thiamin. The ability of the organism to make these will therefore tend also to be lost through mutation. If this happens for both components, at least three mutations will have become established. Even if each is reversible, the probability of all three mutating back to normal simultaneously becomes small. If any one of these genes becomes completely lost, restoration of the synthesis through mutation becomes even more difficult.

If evolutionary loss of synthetic ability is a process so difficult of reversal, it is fair to ask how elaborate systems of reactions leading to the final synthesis of a desired end product ever arose in the first place. If this depended on the simultaneous mutation of genes controlling all steps in the synthesis it seems almost inconceivable that it could have occurred, particularly if totally new types of genes had to arise from existing ones through mutation. If each intermediate compound in the system were independently useful to the organism the series could evolve from the beginning and progress step by step toward the final product, with each successive step having a selective advantage over the preceding one. It seems unlikely, however, that the rather severe restrictions imposed by such a system would be met. Horowitz (25) has recently made the suggestion that if we accept the thesis of Oparin (42) and others that the first

self-duplicating units of life reproduced by taking up preformed relatively complex organic molecules from a medium in which they had spontaneously accumulated, it is tenable to suppose that the evolution of systems of reactions took place backward from the final product. In this way each successive step in the process would make the organism less dependent on organic molecules and would have a corresponding selective advantage. The evolution of a completely autotrophic organism seems at least conceivable on such a basis. An important corollary of this hypothesis of the backward evolution of syntheses is that it is unnecessary to assume that all lines of descent trace back through completely autotrophic ancestors.

As Horowitz points out, many present day parasites live in environments essentially similar to the hypothetical world of free organic molecules in which Oparin assumes life to have originated. It is conceivable that such parasites might under certain conditions reverse their evolution trends and again progress in the direction of autotrophism. However, while this possibility must not be excluded, the probability of its happening must be negligible as compared with the probability of specialization through loss of synthetic ability.

#### Acknowledgements

The author is indebted to Professor F W Went of the California Institute of Technology for calling his attention to Heyne's and Vorderman's descriptions of "*Monilia sitophila*" and for translating relevant passages in their works. Doctor Barbara McClintock of the Carnegie Institution of Washington and a number of the writer's colleagues at Stanford University have permitted reference to observations and ideas that have not as yet been published. For this permission and for helpful criticism the writer is grateful.

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# RELATIVE NUTRITIVE VALUES OF ANIMAL AND VEGETABLE FATS

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The nutritive value of fats has engaged the attention of scientific investigators for many years. More recently, as a result of the war situation, the question of fat supply and best utilization of what is available has resulted in considerable attention being focussed on this problem. Certain fats, notably butter, natural vegetable oils and their hydrogenated derivatives, and the margarines have assumed especial prominence in this connection.

The nutritive value of fats can be considered from several points of view. It is obvious that any fat, in order to serve as food, must be capable of both assimilation and then utilization in metabolism. This involves digestion and absorption. One may therefore consider first of all the extent to which various edible fats are digested and absorbed. Fats are important sources of energy to the body and therefore may be compared with respect to their yield of calories. To a very large extent questions under this heading would be answered by the available data pertaining to the digestion and utilization of different fats. Edible fats also serve certain non-caloric functions. Their value in nutrition can vary according to their composition and content of essential fatty acids, notably, linoleic acid. Edible fats are known to vary in their content of fat-soluble vitamins and this can serve as another basis for estimating their value in nutrition. There are claims in the literature to the effect that certain fats contain unknown fat-soluble dietary essentials remaining to be isolated and identified. Fats, therefore, may be rated according to their content of such unknown factors. Perhaps the most important basis upon which to rest any nutritive comparisons of fats is their value for promoting certain biological functions, notably, those of growth, pregnancy and lactation. The most recent work relating to our theme has centered around a study of this particular topic.

It is pertinent to indicate the limitations of the present discussion. It will not consider (a) the mechanisms and theories of digestion and absorption, (b) the intermediary metabolism of fats, and (c) clinical phases of the topic. For discussion of these subjects the reader is referred to several important reviews, notably that by Anderson and Williams (1), the article by Bloor (2) and that by Burr and Barnes (3), all of which have appeared in this Journal. Another review of interest is a publication of the Council on Foods and Nutrition of the American Medical Association (4). A short review of the most recent work has also appeared in Nutrition Reviews (5). In addition to the foregoing the reader would do well to consult the chapters on fat metabolism that have appeared in various volumes of Annual Reviews of Biochemistry the first of which was published in 1932.

**DIGESTION AND UTILIZATION OF FATS** Studies of the digestion and utilization of various fats have been conducted on several species. The observations

of Langworthy and associates (6) made on students or laboratory assistants are noteworthy. The digestibility of 23 animal, 34 vegetable and 6 hydrogenated fats was studied by feeding amounts ranging from 50 to 150 grams. The fats were incorporated into a corn starch pudding which was fed as part of a simple standard basal ration. Analyses were made for fecal fat. No pronounced differences were noted in the digestibility of the fats studied. There was a suggestion that the thoroughness of digestion was inversely proportional to the melting point in the case of the hydrogenated fats, those hydrogenated to a definite melting point, and those made by mixing soft and hard fats showed no definite differences in thoroughness of digestion. In only two cases was a laxative effect noted, these being cacao butter and goose fat. The illustrative digestibility coefficients for certain commonly used fats are the following: butter fat 97 per cent, beef kidney fat 88 per cent, cocoanut oil 97.9 per cent, coconut butter 95.9 per cent, corn oil 96.8 per cent and soy oil 97.5 per cent. In an earlier report Holmes and Deuel (7) gave data on digestibility coefficients for corn oil, cottonseed oil and peanut oil that had been hardened to varying degrees by hydrogenation. For hardened cottonseed oil with melting points 35°, 38.6°, and 46°, the respective digestibility coefficients were 96.8, 95.5 and 94.9 per cent. Similar data for hydrogenated peanut oil with melting points 37°, 39°, 43°, 50° and 52.4°, were, respectively, 98.1, 95.9, 96.6, 92.0 and 79.0 per cent. Corn oil hardened by hydrogenation so as to have melting points of 33°, 43° and 50° had 94.7, 95.4 and 88.5 per cent as their respective coefficients. In general, digestibility decreases as the melting point increases. All the fats melting below 46° were well utilized. The same authors (8) studied the digestibility of cod-liver, java almond, tea-seed and watermelon seed oils, deer fat and some blended hydrogenated fats. The blended fats with different melting points prepared from corn, cottonseed and peanut oil showed coefficients of digestibility ranging from 91.5 to 97.4 per cent, a blended cottonseed fat that melted at 50° gave a coefficient of 97.0 per cent. The coefficients for cod liver, java almond, tea-seed, watermelon seed oils and deer fat decreased in the order named from 97.7 to 81.7 per cent.

Some observations have been made of the digestibility of fats of different tissue origins. Holmes (9) tested fats obtained from different parts of the body of cattle but not from the same animal. Two rather unusual items were tested, namely, hard palate fat and ox tail fat. Tests were also made of butter and cream for purposes of comparison. The digestibility of brisket fat, ox tail fat, butter and cream proved to be 97 per cent, the value for hard palate fat, kidney fat and ox marrow fat was 94 per cent, for oleo oil and oleostearin the values were 97 and 80 per cent, respectively. An interesting observation was that ox marrow fat caused distressing disturbances in all subjects when the smallest test dose, namely, 59 grams, was ingested daily. The other fats were given in amounts ranging from 59 to 100 grams daily and produced no abnormal physiological reaction.

Corn oil and cottonseed oil have been tested in dogs (10) and found to be 99 per cent digestible.

Holt and associates (11) determined the digestibility coefficients in normal infants and obtained the following results corn oil 96.9, soy bean oil 93.7, coconut oil 88.7, butter 88.9 and a mixture of butter and corn oil 96.6 per cent. It will be noticed that these values are not greatly different from those reported by Langworthy, Holmes and Deuel. In an estimation of the value of various fats for premature infants Tidwell et al. (12) noted that in such infants olive oil and soy bean oil are better absorbed than butter fat.

Steenbock, Irwin and Weber (13) studied the percentage of fat absorbed from the alimentary canal of rats at 2, 4, 6, 8 and 12 hours after feeding definite quantities of fat. It was found that (a) partially hydrogenated vegetable oils, as sold commercially for home and bakers' use, were absorbed as rapidly as lard or corn oil, and (b) that butter oil, halibut liver and cod-liver oil were absorbed uniformly at a more rapid rate than corn oil, lard, or the partially hydrogenated fats. Eleven other samples of fat tested after a 4-hour absorption period could be arranged in the following descending order of their percentage absorption: linseed oil, olive oil, whale oil, soy bean oil, peanut oil, rancid lard, cottonseed oil, cocobutter, cocoanut oil, palm oil and oleo stock. The authors make the statement that "although the difference between any of these fats and the one immediately preceding or following in the list may not be significant, certainly there were very real differences in the absorption rates of those widely separated." Studies of this type using the rat as the experimental animal have also been made by Hoagland and Snider (14). Under the conditions of their experiments there was no consistent relationship between the melting points of the fats and their digestibility coefficients. When the diets contained 5 per cent of fat, the following digestibility coefficients were obtained: cocoanut oil 98.9, soybean oil 98.5, corn oil 97.5, butter fat 88.3, mutton tallow 74.6, oleo stock 74, and cacao butter 63.3 per cent. These results should be compared with those obtained in experiments where the diets contained 15 per cent of fat. In a few instances this feeding of higher level of fat improved the coefficients somewhat, notably those of the fats which rated low when only 5 per cent of fat was fed. The results were: soy bean oil 98.3, corn oil 98.3, cocoanut oil 96.5, butter fat 90.7, oleo stock 86.7, mutton tallow 84.8, and cacao butter 81.6 per cent. In experiments with men, made as part of a study of whole wheat and white breads, Sealock, Basinski and Murlin (15) observed a high digestibility of the fats present in the mixed diets, the digestibilities ranging from 95.7 to 97.4 per cent.

**NON-CALORIC FUNCTIONS OF FAT.** *Fats as sources of essential fatty acids.* Considerable information on this subject has accumulated since the pioneer observations of Burr and Burr (16). Since it was reviewed in 1943 by Burr and Barnes the topic does not need extended discussion here. Let it suffice to quote a summary statement from the Burr and Barnes review (3, p. 261):

Those acids which have been reported to have some curative effect upon fat deficient rats are linoleic, linolenic, arachidonic, decosahexanoic and hexahydroxystearic. In addition, linoleyl alcohol is useful. All other fatty acids and isomers of the above compounds have been reported negative. Linoleic, arachidonic and linolenic acids are the only ones extensively worked with. Quantitative comparisons of their growth effects,

made in different laboratories are not in close agreement but it is clear that linoleic and arachidonic are much alike while linolenic is inferior. This acid produces some growth but does not cure the skin, reduce water consumption to normal or improve lactation (Burr 1942, Quackenbush et al. 1942) (Burr 1942 Fed Proc 1:224, Quackenbush, Kummerow and Steenbock, 1942 J Nutrition 24:213). Decosahexoleic acid and the mixed esters of cod liver oil react in a similar way and it can be said that linoleic and arachidonic acids are highly specific in their effects upon the skin.

The foregoing quotation of course suggests that the distribution of these particular fatty acids in vegetable and animal fats should be ascertained.

One cannot, however, dispose of this problem merely by considering the amounts of these fatty acids present in various fats, because there are observations suggesting that essential fatty acid, pyridoxine, pantothenic acid and perhaps even other factors may in some way be related to the prevention and cure of "acrodynia" in rats. This is illustrated by the striking curative effect of linoleic acid on the acrodynia like disease produced in rats on fat-low diets observed by Quackenbush, Platz and Steenbock (17) who wrote as follows: "Complete healing of either the acute or chronic form was obtained by the administration of peanut oil or wheat germ oil. One-half drop of ethyl linoleate per day cured the dermatitis completely." In another paper (18) this same group of workers confirmed numerous other investigators who had called attention to members of the B-complex group of vitamins being involved in the production and prevention of this experimental acrodynia.

Linoleic acid when fed as the ethyl ester at a subcurative level, viz., 5 mg daily, produced an effect similar to that obtained with pyridoxine. When fed at higher levels, viz., 10 mg or more, the acrodynia was cured completely. Pyridoxine together with pantothenic acid produced a greater improvement than was obtained with pyridoxine alone, but less than linoleic ester or a commercially available rice bran concentrate. Pantothenic acid alone did not even alleviate the symptoms. The three compounds together cured the acrodynia but did not cure completely the scaly condition of the tail and hind paws.

These authors considered their results to indicate that an additional factor is involved.

*Fats as sources of known vitamins.* It has long been appreciated that fats vary with respect to their content of the known fat-soluble vitamins. Numerous tables of food composition have been published which may be consulted for detailed data on this subject. The discussion here will be limited to a few special points of interest.

With respect to vitamin A and its provitamin carotene, the latter and its biologically effective carotenoid relatives constitute the form to be found in plant tissues such as, for example, the leaves of spinach, greens, and alfalfa. The vitamin A potency of butter is due partly to carotene and partly to vitamin A itself. Potent intracellular fats of animal origin, cod liver oil for example, contain vitamin A as such. The fats found in the fat depots of the animal body rate so low in vitamin A content as to constitute an insignificant source of this vitamin for human nutrition. Similarly, the fat depots in plants, oily seeds for example, are, broadly speaking, practically devoid of vitamin A potency. These

are the important commercial sources of such oils as palm oil, cottonseed oil, olive oil, etc. These oils are found in the kernels of the respective seeds, and are to be distinguished from the oils that can be extracted from the outer covering or cortex of the seeds, some of which have been found to be good sources of carotene (19). Rosedale (20) has reported a rather high vitamin A potency for the red palm oil of Malaya, *Elaeis guineensis*. The Indian Research Fund Association has suggested that through more extensive cultivation in India of the palm from which this oil is obtained and education of the Indian population concerning the value of this oil as a food much could be done to improve the vitamin A intake of the people of that subcontinent (21). Buckley (22) has determined biologically the carotene content of this red palm oil as affected by degree of ripeness and noted some differences, oil from the ripe fruit contained 1900 international units of vitamin A per gram, that from over-ripe fruit 1600 I U /gram, and the unripe fruit only 600 I U /gram. Palatability, and concentration of the carotene could be increased by removal of the solid constituents that settle from the oil which is normally liquid at Malayan temperature. The Indian Research Fund Association (21) made a study of the possibilities for use in India, of this red palm oil. The palm *Elaeis guineensis* is native to West Africa. By 1937 it had been introduced into Malaya and Burma but not into India. A clinical trial showed that a suitable emulsion was just as effective as cod liver oil in severe cases of keratomalacia in Madras. It was roughly calculated that in India a given amount of vitamin A potency as red palm oil would cost about one-third of the cost of the same amount of cod liver oil. The oil could be used medicinally or as a culinary fat or, when mixed with cocoanut oil or hydrogenated fat, as a kind of butter or margarine. De (23) fried chipped potatoes in mixtures of red palm oil and various other vegetable oils at temperatures of 100 to 140°C, and found the resulting product to contain substantial amounts of carotene and to be quite palatable. This investigator (24) has also reported on the carotene content of about 80 vegetable foods eaten in India. Observations of the sort just cited could doubtless be made with respect to still other valuable sources of carotene available in various parts of the world, particularly in the tropics, and as yet not used as human food to the extent that their nutritive values warrant. The greater tendency of fats to turn rancid in the tropics probably means that rich sources of carotene constitute the most practical ways for providing vitamin A to the masses living in the tropic zone. This need not be the case for all of the inhabitants in such areas in view of recent researches aimed at providing American troops stationed in the tropics with butter and related fats that have been stabilized, so to speak, so as to be capable of storage for considerable periods without deterioration of any kind. Presumably such newly developed products can be made available in tropical countries, but the amounts provided would doubtless furnish but a small part of the total need of the population. In arctic regions, on the other hand, fish oils play a similar rôle.

In the temperate zone, particularly in the United States and certain other countries that have a well developed dairy industry, butter is one of the most important sources of vitamin A. In recent years many margarines made largely

if not entirely from vegetable oils have been developed and used as butter substitutes. Most margarines in the United States are now fortified with vitamin A, and a standard for such a product has been established by the Food and Drug Administration of the federal government (25). This standard requires that fortified oleomargarine contain not less than 9000 I U of vitamin A per pound. This value was selected after consideration of the data on the vitamin A content of butter that were available at the time the standard was being considered. It is known that the vitamin A value of butter depends upon the feed of the cow, the values are low in the winter season and high when the animals are given access to green pasturage. The most recently obtained data are well illustrated by those published by Berl and Peterson (26) who analysed numerous samples of Wisconsin butters. "March butters averaged 9500 I U/lb. The butters of July and September averaged about 18,000 I U/lb. The January butters were slightly better than the March butters and contained about 10,500 I U/lb. In the summer butters, about 75 per cent of the total natural butter pigment was found to be carotene, in the winter butters 60 to 65 per cent of the pigment was carotene."

With respect to vitamin D, the analyses published by Wilkinson (27) indicate that butter contains from 50 to 500 I U/lb. The margarines are practically devoid of vitamin D unless fish liver oils or their concentrates are the sources of vitamin A used in the fortification. In England both vitamins A and D are added to margarines. According to the compilations given in Miscellaneous Circular 275 of the United States Department of Agriculture (28) natural foods which contain vitamin D are of animal origin. Fish which contain much body oil, such as salmon, herring and sardines, are the richest natural sources, eggs come next, then milk fat, meat products contain some vitamin D.

The richest natural sources of vitamin E are the oils contained in plant embryos. Wheat germ oil is the prime example here. The margarines can therefore contain appreciable amounts of vitamin E depending upon the formula used. Butter has been shown to be low in content of this factor, but the amount present in whole milk has been shown to be sufficient to meet the needs for reproduction in dogs and rats. The significance of vitamin E in human nutrition has not been satisfactorily evaluated. Because of this fact, nutritional claims for vitamin E are as yet not recognized by the Councils of the American Medical Association and by the Food and Drug Administration of the federal government.

With respect to vitamin K little can be said. The amount of this factor in butter has not been determined accurately, but various considerations lead to the conclusion that the amount present must be small. The seeds of plants contain less than do the leafy parts of the plant. This suggests that the margarines should contain only small amounts. In view of the fact that the intestinal bacteria can synthesize all of the vitamin K needed by the body (at least that of adults), any slight differences which plant and animal fats may show in vitamin K content are probably of no nutritional significance.

*Fats as sources of unidentified dietary essentials.* The possibility that unknown dietary essentials are present in certain fats is suggested by the work of Wulzen

and associates which is interpreted to mean that cream contains an unknown factor needed by the guinea pig to prevent a marked stiffness and final rigidity of the wrists. The pioneer observations were made in experiments where planarian worms were fed various tissues of rats and guinea pigs that were deficient in vitamins A or B-complex or C (29). van Wagtenonk and Wulzen (30) finally announced the isolation of a curative "anti-stiffness" substance in crystalline form from raw cream, prepared by distilling fatty acids with steam and separating the active agent in the distillate from acidic compounds, final crystallization involved the preparation of a mercury iodide complex derivative formed with Girard's reagent. The vitamin recovered from this crystalline derivative was a pale yellow oil with ten million times the activity of the cream from which it was prepared. It was claimed that the vitamin is a carbonyl compound with a molecular weight of about 200. A dose of 0.1 microgram per day administered daily for five days sufficed to cure a guinea pig of the stiffness of the wrist joints. The symptoms exhibited by guinea pigs deficient in this "anti-stiffness" factor prove to be similar in some respects to those of lack of alpha-tocopherol and ascorbic acid, and one may raise the question whether the amounts of these dietary essentials in the experimental diet were adequate. In a later paper (31) it is shown, by a comparative study of the creatine excretion of animals on a diet deficient in the new dietary essential and in vitamin E, that these two factors are not the same. "A deficiency of the anti-stiffness factor produces in guinea pigs a characteristic wrist-stiffness but has no significant effect upon the creatine excretion."

A characteristic feature of the wrist-stiffness disease is the deposit of large amounts of calcium, mainly in the form of calcium triphosphate in connection with almost any body tissue, "conspicuous locations being muscle, subcutaneous tissue, aorta, liver and kidneys. This abnormal deposition of calcium phosphate suggested a derangement of the phosphorus metabolism." For this reason a study was made of the distribution of the acid-soluble phosphorus in the liver and kidneys of normal and deficient guinea pigs. The authors stated that "the interpretation of the changes in the distribution of the acid-soluble phosphorus in the liver and kidneys occurring in the course of the deficiency is as yet difficult." It was suggested that possibly "changes in the adenosine triphosphate and the adenosine diphosphate are of prominence during the deficiency, perhaps involving the whole metabolism of the purines." Further studies (32) revealed that along with these changes in the distribution of acid-soluble phosphorus there is a decrease in the alkaline serum phosphatase. Ascorbic acid in sufficient amounts to prevent scurvy did not prevent this drop in serum phosphatase level. On the other hand, "continuous administration of the anti-stiffness factor did prevent the low level."

With respect to the special theme of the present review it is pertinent to point out that the distribution of this factor among many fats has not been reported. Wheat germ oil and cod liver oil were reported to be ineffective. In many of the experiments the fat soluble vitamins were administered as solutions in cottonseed oil, but this cannot be interpreted to mean that the factor is absent from

cottonseed oil because the amount of the oil administered as a carrier of the fat soluble vitamins was undoubtedly small, it could be present but in an amount too small relatively to be significant in the experiment

**EFFECT ON THE REQUIREMENT OF OTHER NUTRIENTS** The "vitamin B<sub>1</sub> sparing action" of diets high in fat is one illustration of the way fats can influence the requirement of other nutrients. The early observations of this phenomenon by Evans and Lepkovsky (33, 34) were made on rats fed diets containing as the fat component either lard, cottonseed oil (Wesson), partially hydrogenated cottonseed oil (Crisco), butter, corn oil, cocoanut oil and walnut oil. The authors stated (34) that "all natural fats studied by us have shown the sparing action on the antineuritic vitamin B". It will be noted that the list just cited includes both animal and vegetable fats. The observations of Arnold and Elvehjem (35) were made on dogs fed diets in which the fat components of interest were autoclaved lard and cottonseed oil, one an animal fat, the other a vegetable oil. Stern, Arnold and Elvehjem (36) experimented with growing rats and used butter fat, cocoanut oil, cottonseed oil, olive oil, peanut oil, and a group of autoclaved natural fats which included lard, peanut oil, cod liver oil and cottonseed oil, synthetic tricaproin and triacetin were also tested. All of the fats and oils showed this vitamin sparing action, no marked differences among them were noted. To this list of fats should be added certain other substances studied by Salmon and Goodman (37), namely, pecan oil and numerous synthetic esters of single fatty acids. These workers considered that cocoanut fat was the most effective natural fat tested in high fat diets with respect to effect in increasing the rate of growth and decreasing the incidence of beriberi in rats. "The effectiveness of esters of single fatty acids in alleviating the symptoms of vitamin B deficiency in rats depended upon the length of the carbon chain of the fatty acid. The effectiveness was maximum at the 8-carbon acid and decreased in each direction from this point". Much of the early work on this problem was complicated by the observation that the amount of autoclaved yeast used in the diet was a factor, also, the importance of essential fatty acid was not always realized, and therefore lack of this factor may have played a rôle. In the papers cited in this review the interested reader will find references to further literature dealing with these various points.

It is possible that high levels of fat in the diet increase the requirement for riboflavin. Mannering, Lipton and Elvehjem (38) fed groups of growing rats diets in which lard was isodynamically substituted for dextrin at two levels to give rations the total fat contents of which were 25 per cent and 40 per cent respectively. Different groups of young rats subsisted on these rations supplemented with 3 micrograms and 6 micrograms of riboflavin. Increasing the fat level in a riboflavin low ration adversely affected the growth of the animals, the administration of adequate amounts of riboflavin completely corrected the deficiency. The authors discussed possible explanations of this phenomenon. "Increasing the fat level in the diet may alter the intestinal flora so that less than normal bacterial synthesis, or more than normal bacterial destruction of riboflavin occurs or riboflavin may be directly concerned with fat metabolism



or phosphorylation " It should be noted that only lard was used in these particular experiments Presumably the Wisconsin workers have made additional unpublished observations with still other fats, for in a review article by the Council on Foods and Nutrition of the American Medical Association (4) this study is referred to and the following statement made "In the rat, high levels of fat appear to increase the riboflavin requirement, but again all fats function equally " Observations on puppies, on the other hand, do not support such a conclusion Potter, Axelrod and Elvehjem (39) reported the daily riboflavin requirement of the growing dog to be from 60 to 100 micrograms per kilogram bodyweight, and that the isocaloric substitution of lard for sucrose did not increase it In these experiments the high carbohydrate diet contained 66 per cent sucrose, 8 per cent cottonseed oil and 3 per cent cod liver oil, the high fat diet was made by isocalorically replacing a portion of this sucrose with lard It seems evident that further work is needed on this problem before a general relationship between level of fat in the diet and riboflavin requirement can be regarded as demonstrated

Some reference should be made to what might be called the pyridoxine-sparing action of fats In the early literature on vitamin B<sub>6</sub> one can find many instances where investigators found it more difficult to produce the typical picture of pyridoxine deficiency when the diet contained much fat, the feeding of a low-fat ration proved to be an important factor in such experiments The work of Quackenbush et al (18), already discussed, was interpreted in favor of the view that linoleic acid, pyridoxine, pantothenic acid and some additional factor are all involved in the cure of rat "acrodynia" It seems probable, therefore, that the rôle played here by any particular natural fat, whether of plant or animal origin, is related fundamentally to its content of essential fatty acid, particularly linoleic acid It also seems evident that further studies of these relationships between essential fatty acid and certain B-complex vitamins are needed

Choline is now known to be an important dietary factor One of the significant features of choline deficiency in animals is fatty liver This suggests that in any consideration of the relative nutritive values of plant and animal fats, possible relations to choline requirement should receive attention The production of this particular form of fatty liver, however, is apparently related to a need for choline to assist in removing fat from the liver to fatty depots, to the extent that natural fats furnish phospholipids containing choline, such fats contribute something in meeting this need It becomes of interest in this connection, therefore, to consider the distribution of choline in plant and animal products According to Engel (40), "animal organs, egg yolk and nervous tissue were found to be considerably better sources of choline than any of the plant material examined Green leafy plant material compared favorably with muscle tissue Good sources were found to be green leafy and leguminous vegetables, seed oil meals and grain germs Seed oil meals were found to be equal or superior to the whole seeds skim milk powder contained nearly a third more choline than whole milk powder " Careful examination of his data reveals that lard, cod liver oil, butter, hydrogenated cocoanut oil, oleomargarine and refined corn

oil, refined soybean oil all contained extremely small amounts of choline, actually less than 0.05 mgm./100 grams of fresh material. The significance of this figure is made evident by comparison with the following illustrative ones: beef liver 630, beef roundsteak 95, rolled oats 151, white flour 52, whole milk powder 107, fresh milk 14.7, potatoes 106. It is clear that many foods, cereals and skim milk for example, which in themselves are quite low in fat contribute most of the choline present in the diet along with other lipotropic substances. In the refining of natural fats phospholipids are almost entirely removed, and there are no significant differences between plant and animal fats in this respect.

Interesting observations relating fat to the retention and utilization of the galactose found in ingested milk sugar have been reported by Schantz et al. (41). Rats fed a diet of mineralized whole milk made efficient use of all the lactose as shown by the presence of only a trace of reducing substances in the urine and the absence of reducing substances from the feces. On the other hand, when skim milk was used instead of whole milk, after a few days the urine was found to contain appreciable amounts of reducing substances and the presence of significant amounts of galactose was definitely proven. Experiments showed that the 3 to 4 per cent of fat naturally present in the milk is necessary if the animal is to make complete utilization of the galactose. The milk fat could be replaced by other fats such as lard, corn oil, linseed oil, cocoanut oil, hydrogenated cocoanut oil, triolein (synthetic), tripalmitin (synthetic) and oleic acid. The following substances were not effective in this respect: tricaproin (synthetic), sodium lactate, sodium butyrate, beta hydroxybutyric acid and choline. The list of substances tested was extended by Schantz and Krewson (42).

The triglycerides of caproic, caprylic, capric and lauric acids were synthesized and then homogenized into fresh skim milk at a level of 4 per cent. Within a test period of ten days caproin, caprylin and caprin were not effective, but laurin was. These same animals that were losing galactose via the urine when fed these ineffective fats, began within 3 or 4 days to retain the galactose when corn oil replaced these fats in the skim milk. The 18 carbon ketonic acid, heanic acid, was also tested and found to be effective. The odd chain fatty acid pentedecylic acid was not effective. The data support the generalization that even chain fatty acids containing 12 or more carbon atoms when fed with skim milk to the extent of 3 to 4 per cent are effective, those with less than 12 carbon atoms are not. It will be noticed that this interesting property of affecting the utilization of galactose is not related to the plant or animal origin of the fat but rather to a group of fatty acids that occur widely in natural fats.

*Relative values for growth* This subject has recently had active investigation in several quarters and a controversy has developed regarding the interpretation of the several groups of data. Schantz, Elvehjem and Hart (43) performed experiments with groups of 21-day-old rats fed diets consisting of mineralized skimmed milk into which 4 per cent of fat had been added by homogenization. The respective diets contained as the variable fats of interest, butter fat, corn oil, cocoanut oil, cottonseed oil and soy bean oil. During the first few weeks of each experiment the rats receiving butter fat made better and more efficient gains in

body weight than did the animals eating the other fats, but this difference tended to disappear thereafter. In a later paper (44) experiments with fatty acid fractions of butter fat were reported. The respective fractions were the volatile, the unsaturated and the saturated acids. These were converted into their triglycerides and used as supplements to a diet of corn oil homogenized into the same mineralized and vitamin-supplemented skimmed milk. The animals receiving the acids representing the saturated-acid fraction exhibited a growth rate even greater than that observed when butter fat was the test fat. This claim was given further support by the data reported by Boutwell et al (45) who stated that complete hydrogenation of the unsaturated acid fraction markedly increased its value for growth. These authors chose to regard their observations as favoring the view that there was present in the nonhydrogenated unsaturated acid fraction a long chain fatty acid (or acids) in unsaturated form which was rendered peculiarly effective for growth by hydrogenation, and that this material was apparently not present in either its saturated or unsaturated form in the various other oils tested because hydrogenation of these other oils did not improve their nutritive value when tested by incorporation into the mineralized and vitamin-supplemented skimmed milk. In this connection should be mentioned the claim by Boer (46) that butter fat contains a specific growth-prompting factor because his rats receiving butter fat grew better than those getting olive oil. On the other hand, Euler, Euler and Saberg (47) compared butter and margarine in diets otherwise adequate and observed more rapid growth in the rats subsisting on the margarine rations, these animals also had better hair coats and seemed to be more vigorous.

The next development of interest reported by the Wisconsin group of workers was that the nature of the carbohydrate in the ration plays a rôle. When the sole carbohydrate in the diet was lactose, animals receiving butter fat showed growth superior to that exhibited by the rats receiving corn oil (48, 49). When the carbohydrate was dextrose, sucrose, dextrin or starch, the growth of the corn oil groups was comparable to that of the butter fat groups or even better. The ration employed for these comparisons contained 32 per cent carbohydrate and 28 per cent fat. It was observed that, considered as a group, all of the lactose diets gave the poorer growth. This was explained by the assumption that lactose, as compared with the other carbohydrates, in some way adversely affects the intestinal flora, and that butter fat in some manner counteracts this effect either by supplying an essential growth factor—the unsaturated fatty acid or acids mentioned above—or indirectly by stimulating the bacterial flora to produce some unknown essential factor or factors. There is quite a literature available showing that the nature of the carbohydrate in the diet can affect the amounts of various members of the B complex found in the feces. Therefore the essential factor (or factors) being produced in accordance with this hypothesis might well be water-soluble vitamins instead of fat-soluble substances. These investigators also claimed that the younger the rat was when placed on the lactose-containing diet, the greater was the beneficial effect of the butter fat as compared with the vegetable oils.

In another paper these authors reported on comparisons of butter fat with many other fats and oils including several margarines (50). In these experiments the carbohydrate fraction was increased to 48 per cent because "unpublished work" showed that as the level of lactose was increased, the difference in growth rates between rats fed butter fat and those fed corn oil became greater. It pointed out that "this level of lactose is between the 32-40 per cent found in cow's milk and the 50-55 per cent found in human milk (dry basis)". For comparison with the lactose-containing ration there was used a diet containing a mixture of carbohydrates "modeled after the mixed carbohydrate diet such as man ordinarily consumes, and the results of this work led to an investigation of oleomargarines on similar rations". In all cases the animals on the mixed-carbohydrate rations, regardless of the fat used, exhibited better growth than those on the lactose diets. When lactose was the sole carbohydrate in the ration butter fat and lard were judged to be superior on the basis of the average weights of the six rats in each group at the end of 5 weeks on the diet. The authors did not give any data on the degree of variation characteristic of these averages, nor did they report any calculations made in an effort to confirm their impression that the differences cited as being significant really were significant. Some of the differences shown in their published graphs and tables seem, by mere inspection, to be large enough to be significant, whereas others are of questionable significance. In a matter of this sort the experienced critical judgment of the investigator deserves of course some weight. In view of the diametrically opposite results published by Deuel and others, however, these points of possible criticism seem worthy of note here.

Deuel and associates (51, 52) performed similar experiments with rats fed a diet of mineralized skimmed milk to which test fats were added and failed to observe any significant differences in the growth rates of groups of animals when seven different fats were used, namely, butter, margarine, corn oil, cottonseed oil, olive oil, peanut oil and soybean oil. In these experiments the diet contained 70.6 per cent mineralized skimmed milk, and 29.4 per cent test oil or fat. It will be noticed that the only carbohydrate in the ration was lactose. If the claim of the Wisconsin group of workers is correct, this should have resulted in the observation that butter fat is superior to the other tested fats, but such was not the case. The diet was supplemented with fat-soluble vitamins A, D and E, diacetyl, the substance shown by van Niel et al. (53) to be largely responsible for the characteristic aroma of butter and commercially available for use as a flavoring agent, was added to the various fats to secure equal intakes of the respective diets. These authors make the point that in the experiments by Schantz et al. (43) only carotene was used as a source of vitamin A with the vegetable oils, whereas the animals eating the butter fat diet must have received some vitamin A as such. To the extent that individuals are known to vary in their ability to utilize the provitamin carotene this criticism has merit. On the other hand, if the supply of carotene was very liberal, the animals doubtless secured sufficient vitamin A from this source and therefore this difference in the two sets of experiments can hardly account for the difference in the results.

Clausen, Barnes and Burr (54) have made interesting observations relating rate of growth to the rancidity of the diet that may have a bearing on this controversy. They were led to study the oxidation of the fat contained in experimental animal diets by the observation "that rats receiving a normal balanced diet containing the pure B vitamins in place of the customary yeast, cod liver oil, and lard, failed to grow and ultimately died. Replacement of the lard with other fats such as hydrogenated vegetable oils, corn oil, and butter fat provided an adequate diet for growth. If the pure B vitamins were replaced by yeast, normal growth resulted. On further investigation it was found that in the absence of the yeast, the lard and cod liver oil rapidly became oxidized and exerted a deleterious effect." These authors made the further interesting comment that "the inadvertent destruction of dietary essentials and the possible independent toxicity of rancid fat are factors that might confuse the interpretation of many dietary experiments." They definitely recommended that experimental diets be stored at low temperatures. In the experiments by Deuel et al (52) great care was taken to exclude all rancid fat. The diets were prepared weekly and the unused supply kept in a refrigerator.

The question of the actual amounts of food eaten by the rats is important in studies of the type under discussion. Special efforts were made by Deuel and his collaborators to secure accurate food intake data. These authors criticized the Schantz, Elvehjem and Hart (43) study on the ground that the fat tended to separate out from the skimmed milk and therefore the composition of the food actually eaten at different times of the day was not the same. In their work reported in a later communication, however, Boutwell et al (49) used dry artificial rations, such a criticism would not apply to this study. In feeding experiments involving butter one has to consider the extent to which the food intake is affected by the flavor of this food. Boutwell et al (45) pointed out that the lower growth rate of the animals fed the corn oil diet as compared with those subsisting on the butter or butter fat fractions was associated with a smaller daily consumption of food. These authors chose to interpret this increased appetite and greater palatability as indicating a superior food. This was challenged by Deuel and associates (52) on the basis of the observation that rats "overwhelmingly prefer a diet of peanut oil to which commercial butter flavor has been added to the unflavored" peanut oil ration, and conclude that it is flavor rather than nutritive value that is determining the choice here. These observations on flavor were reported in detail in a later paper (55). Groups of 21-day old rats were given a chance to choose between two food mixtures over periods of 3 to 12 weeks. In the first series of tests the butter diet and one of the other rations containing the other fats (corn, cottonseed, olive, peanut or soy bean oils or margarine) were offered simultaneously. In every series of tests the butter diet was consumed in the larger amount. It was demonstrated that in the majority of instances rats prefer a complete diet the fat of which is butter to one where the fat is replaced by vegetable oils. When the choice was between fats flavored with diacetyl only and those containing commercial butter flavor of which diacetyl constituted 12 per cent, the latter was preferred. Tests with a labora-

tory prepared butter flavor containing monobutyrin, butyric acid and diacetyl to simulate commercial butter flavor gave similar results

As a rebuttal to this argument that it was the preference of their weanling rats for the butter flavor, when fed ad libitum, with consequent greater intake of the butter diet that explained their finding that butter is a superior fat, Boutwell et al (56) cited the results of experiments involving the removal of flavor from butter fat by chromatography or the addition of diacetyl to corn oil. Four diets were used, containing one of the following as the fat portion: (a) butter fat, (b) corn oil, (c) chromatographed butter fat, and (d) corn oil containing 0.0005 per cent diacetyl. When lactose was the sole carbohydrate, and the animals were fed ad libitum, the growth and appearance of the albino male rats was superior to that of the animals fed corn oil. Removal of the flavoring agents from butter, or the addition of diacetyl to corn oil, had little effect on the comparative nutritive value of the two fats. Comparisons of these fats using diets containing dextrose as the sole carbohydrate resulted in no difference, but in this experiment flavor was not the variable of interest. In their discussion these authors criticized the view that the paired feeding technique has special merit and application in studies of this sort. They point out that paired feeding "penalizes the superior food and ultimately obliterates the difference as measured by the growth rate and well being of the experimental animal." This would doubtless be their comment on the study reported by Zialcita and Mitchell (57) who compared butter and corn oil with respect to growth promoting value for baby rats paired according to litter, sex and body weight. The rats were forced fed through a medical syringe adapted for the purpose. Because Boutwell et al (48) had claimed that the younger the experimental animals the greater the differences obtained, Zialcita and Mitchell weaned one group at the age of one week and another at two weeks. Up to the age of three weeks they were fed an artificial liquid milk simulating in composition that of rat milk. One member of each pair received 15 per cent butter fat while the other received 15 per cent corn oil. After weaning age an artificial solid diet was used based on skimmed milk powder supplemented with casein, salt mixture and vitamins and containing 27 per cent of butter fat or corn oil. When the data were submitted to statistical analysis the differences were found not to be significant. The point made by the Wisconsin workers about paired feeding operating to "obliterate the difference as measured by the growth rate" is readily illustrated by the data shown in the tables published by Zialcita and Mitchell. In the series of ad libitum feeding trials, the rats fed corn oil made better gains than those fed butter fat, however, the food consumption was likewise considerably greater. Zialcita and Mitchell concluded that "apart from differences in vitamin content, corn oil and butter fat are essentially equal in growth promoting value for the rat." Deuel and Movitt (58) also performed some experiments with rats weaned when 14 days old. The growth of their animals was followed for a period of 12 weeks. The diets used consisted of the mineralized skimmed milk powder to which was added vitamin fortified corn, cottonseed, peanut or soy bean oil, or whole butter or whole margarine. It was found that the efficiencies of conver

sion of these diets to body tissue were similar. Moreover, the growth rates were similar to those of rats weaned at the usual age, namely 21 days. The prematurely weaned animals attained essentially the same bodyweights at later ages as normally weaned rats, thus showing that the premature weaning exerted no adverse influence.

The importance of considering the degree of variation characteristic of averages and subjecting data to mathematical analysis to determine significance of differences is further illustrated in the work of Freeman and Ivy (59) who observed during the first seven weeks of experiment better growth of weanling rats fed butter fat than of those receiving cocoanut oil, but this difference was not statistically significant, after three months, however, the butter fat fed rats were definitely larger. The observations of Harris and Mosher (60) can be regarded as confirming this "age effect." These investigators placed 120-day old rats on diets differing with respect to fat, one group getting cocoanut oil and the other group butter fat. The experimental diet was based on extracted milk powder, extracted yeast and the test fat which last-named item constituted 25 per cent of the basal mixture. Minerals and vitamins were also provided. The reader will notice that lactose constituted almost all of the carbohydrate in this ration, the amount of other carbohydrate furnished by the dried yeast must have been very small. The food was fed ad libitum. For the first 30 days the growth rates were practically the same. By the 60th day, however, the cocoanut oil-fed animals showed a slightly greater average gain. It is unfortunate that these authors gave no indication of the degree of variation characteristic of their published averages. It seems probable that the small difference noted was significant, but the critical reader would like to have more assurance on this important point.

Deuel and co-workers (52) offered as one of the possible explanations for the difference between their results and those reported by Schantz et al (43) their own use of more animals in a group—at least 14—and their own great care in distributing members of a litter among the various groups to be fed the different test diets. In their work "there were three litters where the rats consistently gained far under the average on the various diets. When a small number of animals is used, variations might be noted especially if two from such a litter were assigned to one group." If some indication is given of the "spread" of the averages, any variation due to the use of such litters would receive some consideration in arriving at conclusions indicated by the data. It seems reasonable to assume that in the work of the Wisconsin group members of litters were properly distributed. On the other hand, the Wisconsin publications fail to indicate the "spread" of the averages and the differences are not subjected to statistical analysis, with the result that the independent critic is unable to determine for himself whether the various differences cited represent what might be expected as chance variations or are significant differences to be rightfully attributed to the presence or absence of lactose or the particular fat used.

If butter fat contains some unknown important factor as claimed by Schantz et al (44) and Boutwell and associates (45), then this may have been present in all of the diets used by Deuel and co-workers, because these investigators used

unextracted skimmed milk powder This could explain why Deuel et al noticed no superiority of butter fat over the other fats and oils tested The skimmed milk powder used by the Wisconsin workers was fat-free, their other rations which did not contain milk powder were purely artificial—so-called synthetic—combinations of food materials This “explanation” has been challenged by Deuel (61) who points out that in the earliest tests reported by Schantz et al (44) liquid skimmed milk was employed, and therefore no such extraction of lipids was employed, yet it was in these experiments that the most marked variations in growth were reported as occurring during a period of three weeks Deuel calculates that the total daily intake of the hypothetical saturated fats of high molecular weight from the unextracted lipids of skimmed milk powder could scarcely exceed 10 mgm “Since most of these fatty acids in this fraction are identical with those present in vegetable fats, those acids characteristic for butterfat and at present not identified would necessarily be present in extremely minute amounts.”

Some observations pertinent to the theme under discussion have been made on calves Gullickson, Fountaine and Fitch (62) fed groups of such animals, 2 to 6 in a group, for periods ranging from a few days to as long as six months The following fats and oils were compared butterfat, lard, tallow, cocoanut oil, peanut oil, corn oil, cottonseed oil and soybean oil Each oil or fat was added to skimmed milk and the mixture then homogenized to yield a product containing 3.5 per cent fat A control group received unhomogenized whole milk. Sufficient cod liver oil to supply vitamins A and D was given separately In growth rate and appearance the whole milk group was definitely superior to the others, with the lard and tallow groups giving almost as good results, the groups receiving cocoanut oil and peanut oil came next in the rating, corn, cottonseed and soybean oils gave definitely unsatisfactory results, some of the calves in these groups not surviving the experiment and others being saved only by transferring them to the whole milk ration

Another factor that may have a bearing on this controversy is the strain of animals used It has been shown that strain differences exist among rats with respect to numerous biological characteristics The “Yale” strain of rats has a relatively low tolerance for glucose (63, 64, 65), the adrenals of male rats of the “Yale” strain are significantly heavier than those of male “Wistar” rats (66), sodium chloride improves the low tolerance to glucose of the “Yale” strain (67)

There are strain differences in rats with respect to the time of onset and degree of incidence of cataract following the feeding of lactose (68) Still other illustrations might be cited but it is unnecessary to list them here. One that is of some interest in relation to the theme under discussion has been reported recently by Erschoff and Deuel (69) Male and female rats of the “Long Evans” and the “U.S.C.” strains were placed at weaning on purified diets containing lactose, beta lactose glucose, galactose, sucrose and corn starch as the sole source of carbohydrate The animals failed to survive on the rations containing lactose or beta-lactose, those of the “U.S.C.” strain living significantly longer, however, than those of the “Long Evans” strain These animals on the lactose



diets developed alopecia, their length of survival was correlated with severity and diarrhea, and a relationship was observed between degree of mortality and previous maternal diet. In discussing the significance of their findings Ershoff and Deuel cite literature dealing with the known effect of dietary lactose on the intestinal flora and the bacterial synthesis of vitamins of the B complex group, and the possibility that the change of the flora to the acidophilic type precipitates nutritional deficiencies either through failure of intestinal synthesis or utilization of factors already present. Boutwell et al. (48) and Geyer and associates (70) had suggested essentially the same possibilities. Examination of earlier diets in which lactose or beta-lactose served as the sole carbohydrate has revealed, according to Ershoff and Deuel,

"the fact that in almost every case natural sources of the vitamin B complex were employed in addition to unextracted casein and fats such as lard or Crisco. The possibility of nutritional factors being present in these materials in sufficient concentration to make good any deficiency resulting from alterations in the intestinal flora has not been excluded. The findings of Boutwell et al. (49, 56) and Geyer et al. (50) that butter fat and lard are superior to corn oil in promoting growth of rats receiving lactose as the sole carbohydrate would seem to indicate this very condition. It seems significant that Geyer et al. (50) reported their rats maintained on the 48 per cent lactose and 28 per cent corn oil ration as developing 'rough and discolored fur coats, blood-stained noses and scaly paws (when the humidity was abnormally high)' "

Similarly, Boutwell et al. (56) noted the occurrence of alopecia in rats eating a similar ration. The Wisconsin workers have stressed the point that more marked differences in growth due to fat are seen when high levels of lactose are fed, but it is at these high levels of lactose-feeding that the phenomena indicative of lack of some members of the B complex most readily occur, and, in view of the Ershoff and Deuel (69) observations, strains of rats can differ significantly in their ability to subsist on high-lactose rations without harm.

It seems evident that only further work on this problem by different groups of workers using the same rations, strains of animals, and as nearly the same experimental conditions as is possible can resolve this interesting controversy.

*Relative values for pregnancy and lactation.* The earlier literature on the relation of fat to lactation was reviewed in 1937 by Anderson and Williams (1). Much of it deals with the relation of dietary constituents to the production of milk in cows, goats and sheep, and the chemical composition of that milk. It has long been known that the food carbohydrate is a source of milk fat. Fat is also a suitable material, and within certain limits the percentage of fat in the milk is influenced by its content in the feed. Beger (71) showed that the feeding of butter fat to goats is associated with increases in the fat content of the milk. Allen (72) found that the feeding of whole milk to dairy cows in addition to the usual ration results in a marked increase in the yield of butter fat. The work of Williams and Maynard (73) on lactating goats emphasized that the feeding of a fat-free ration is associated with a rapid drop in milk yield and an equal or greater decline in fat yield, the effect of adding a fat supplement was always to arrest wholly or in part this decline, although butter oil and cocoanut oil increased the

fat content. The lack of fat per se thus appears to be an important factor. Anderson and Williams (1) make the statement that "much of the work on the feeding of palm kernel oil, cocoanut oil and butter fat to lactating animals lacks significance due to the fact that the augmented fat content of the milk resulted from lowered milk yield rather than to the influence of increased dietary fat." Maynard (74) has suggested a reason why the feeding of cocoanut oil meal and palm kernel oil meal high in fat can raise the fat content of the milk as reported by certain investigators. "These fats are rather unique as regards fatty acid distribution in containing, as does milk fat, a considerable amount of acids lower than  $C_{14}$ , though not containing the very low ones present in milk fat. Since butter itself is effective, perhaps a fat with a fatty acid distribution approaching it in some respects would have some effect."

In their series of papers dealing with the comparative nutritive values of fats Deuel and associates have one (75) reporting observations concerning fertility and lactation in animals that had been raised on various diets consisting of mineralized vitamin fortified skimmed milk powder containing butter or on similar diets containing corn, cottonseed, olive, peanut or soy bean oil, or a margarine in place of the butter. No flavoring was added to the rations fed to the first series of animals. When the importance of flavoring became evident—discussed earlier in this review—diacetyl or commercial butter flavor was added to the rations used in the later series (II and III). The various diets were adequate for fertility shown by the fact that 94 per cent of the females cast litters. These authors comment "If fat per se is required for fertility and pregnancy, its requirement may be equally well satisfied by the various vegetable fats as by butter. From the standpoint of lactation, as judged by the survival of the litter and the weight of the young at weaning time, the different fats were also equally effective." Olive oil represented a possible exception to this last statement, but because of the small number of rats receiving this fat, no conclusion is really justified with respect to it.

Maynard and Rasmussen (76) published observations on the influence of certain dietary fats on lactation performance in rats. Two experiments are reported. An application of the paired feeding technic to the problem was devised. It is worth describing here. The mother rats had been fed a stock diet from weaning as well as through gestation. After the birth of the young, pairs of mothers were chosen which were equal in weight, and from whose litters six young for paired groups of nearly equal body weight could be selected. When ever possible these paired groups were equalized as to sex. One mother was fed the high fat diet, and the other, the low fat diet, equalizing the caloric intake in accordance with the appetite of the one consuming the smaller amount. This proved to be the rat on the low fat diet in almost all cases. The young were weighed daily for 17 days to provide growth records during the period when they were entirely dependent upon mothers' milk. This furnished a measure of lactation performance. The young were then sacrificed and analysed. In the first experiment 7 per cent of corn oil was the fat added to the low fat ration to make the high fat diet. In the second series a purified artificial low fat diet was de-

vised, in order to make the high-fat diet, 15 parts of the corn starch were replaced by either crude cocoanut oil or crude corn oil. In both experiments the young from the mothers on the high-fat diet made better growth and contained more dry matter, fat and protein. The markedly different fatty acid make-up of corn oil and cocoanut oil had no detectable influence on the results. These findings agree with those reviewed earlier by Anderson and Williams (1) and already discussed. They have been extended by the work of Loosh et al (77) who used the same technic and compared a fat-free diet with rations containing varying amounts of corn oil and hydrogenated cocoanut oil. The young suckled by mothers fed the corn oil diet made more rapid growth than those whose mothers ate the fat-free ration. Carcass analyses showed that the extra gain of the young consisted largely of fat. The diet containing hydrogenated cocoanut oil gave no better growth than the fat-free diet, and thus could not be remedied by feeding ethyl linolate to the mothers or directly to the young. Similarly, feeding the mothers 125 mg of ethyl linolate each day when they were eating the fat-free diet did not improve the lactation response. The adverse effect of hydrogenating the cocoanut oil was therefore not due merely to saturation of any essential unsaturated fatty acids. This observation of poor performance with hydrogenated cocoanut oil does not agree with the results obtained by Deuel et al (75) discussed previously. No obvious explanation is evident. It is obvious, however, that the technics employed were not quite the same.

Vinson and Cerecedo (78) have recorded some observations on growth, reproduction and lactation in rats maintained through four generations on highly purified diets. A diet containing 10 per cent fat in the form of corn oil was totally inadequate in supporting lactation, with hydrogenated cottonseed oil (Crisco) and lard present to the extent of 15 per cent of the ration, lactation was regarded as good and growth of the young considered to be normal. It was possible to get normal growth with the diet containing 20 per cent protein (casein) and 10 per cent lard, but this did not support lactation, when the protein was increased to 30 per cent, adequate lactation was obtained. During lactation on these purified diets the mothers lost considerable weight and this could be prevented by the feeding of yeast in contrast to the ineffectiveness of administration of biotin, para-aminobenzoic acid plus inositol, and yeast nucleic acid. The requirements of various specific factors for lactation still remain to be determined and the literature on this subject need not be reviewed here. It will be noticed that the levels of both fat and protein in the diets employed by Vinson and Cerecedo were shown to be important in relation to lactation performance. The experiments described were not devised to furnish a test of various fats with respect to nutritive value.

In the latest paper of their series Deuel and associates (79) report observations of successful growth and reproduction of rats over ten generations in which the lineage was through the first litter and of eight generations in which the lineage was through the second litter, where the diets were a modification of the Sherman diet B in which butterfat was replaced by vitamin A-fortified margarine fat. The Sherman B diet consists of two-thirds whole wheat and one-third whole milk powder. On such diets the growth rate considerably exceeded that ob-

tained with animals on a stock diet and progressively improved with the later generations. Sherman and Campbell (80) have reported the ability of their B diet to support excellent growth of the young, maintenance in health of the adults, and the requirements of reproduction including lactation over many generations, a decrease in the infant mortality observed with the A diet (consisting of one-sixth whole milk powder and five-sixths whole wheat), and an increase of 10 per cent in the average length of life of the adults compared with that observed with the A diet. Obviously the B diet is an excellent one. Deuel et al (79) concluded "that a vegetable fat such as that contained in a margarine can serve adequately in place of butter fat for growth and reproduction on a diet otherwise nutritionally satisfactory." These authors considered that their 'results answer in the affirmative the question raised by Boutwell et al (48) as to the adequacy of a vegetable fat for continued growth and reproduction over a number of generations." To this reviewer it seems pertinent to point out that, as shown in the paper, these results were obtained with a diet containing extracted skimmed milk powder and the diet was supplemented once weekly with 5 grams each of lean beef and lettuce. The conclusion reached by these authors as stated above, is doubtless justified especially when the qualifying words "on a diet otherwise nutritionally adequate" are used and when one considers the varied dietary conditions pertaining to man that commonly prevail. Experiments of this sort involving the use of the Sherman B diet or modifications of it, however, can hardly be regarded as critical in nature in resolving the controversy raised by the claim that butter fat has a special value in nutrition when lactose is the sole carbohydrate in the diet. Their significance for practical nutrition is quite evident however.

#### SUMMARY

Edible fats the melting points of which are not too high to prevent liquefaction in the alimentary tract are digested and absorbed to about the same degree. Such differences as have been found are of no practical nutritional significance.

Edible fats differ with respect to their value as a source of known vitamins. Fish oils contain vitamin A as such, the vitamin A potency of butter is due partly to vitamin A and partly to the provitamin carotene, the vitamin A potency of plant oils is an expression of their content of carotene and related biologically effective carotenoids.

With respect to vitamin D, butter contains variable amounts, the margarines are practically devoid of it unless fish oils containing this factor are used to supply vitamin A for fortification, natural foods which contain vitamin D are of animal origin.

The oils of plant embryos are the richest natural sources of vitamin E, the amount of this vitamin in margarines depends upon the formula used, butter contains small but significant amounts.

Little can be said regarding the amounts of vitamin K present in plant and animal fats, any slight differences are probably of no nutritional significance. It has been claimed that cream contains an unknown factor essential for the guinea pig.

Natural fats differ with respect to their content of the essential unsaturated fatty acids but the amounts needed by the organism are so small that these differences are probably of no practical nutritional significance

Natural fats have not been found to differ appreciably in their effects on the body's needs for other dietary essentials

With respect to the value of various fats for growth evidence has been offered by one group of investigators that when lactose is the sole carbohydrate in the food, butter fat is slightly superior to margarine and various oils of plant origin but this view has been vigorously challenged by other workers, with diets similar to the so-called mixed diets used by man such fats have essentially the same nutritive value The lack of fat per se results in lowered milk production by the lactating animal There is some evidence that lactation performance is not as good when the organism is fed hydrogenated cocoanut oil as compared with that when the natural cocoanut oil is fed, this difference is not obviated by supplying essential fatty acid In a diet otherwise nutritionally satisfactory, a vegetable fat such as that contained in a margarine can serve adequately in place of butter fat for growth and reproduction, shown by experiments with eight and more successive generations of rats

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# INTERFERENCE WITH BIOLOGICAL PROCESSES THROUGH THE USE OF ANALOGS OF ESSENTIAL METABOLITES

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Until quite recently the study of the relation of chemical constitution to biological action proceeded along pathways well established by precedent. Observations made during routine testing of compounds, or often by chance, served as the basis for the synthesis of various types of chemically related substances. In this manner many substances of great practical utility were found, and in the same manner many more will undoubtedly be found in the future. Also, through this type of approach, much information has been accumulated which is significant to the correlation of pharmacological action with various chemical and physical characteristics in series of compounds. Only rarely, however, has information so acquired been sufficient, or has our knowledge of the mechanism of action proved adequate, to permit the purposeful design of new compounds of greater effectiveness. These empirical methods of approach, although by no means unfruitful, are tedious and only slowly productive of results, it is encouraging to find, therefore, among recent studies, evidence that elements of logic may now be admixed with empiricism.

The development of these newer concepts stemmed directly from the discovery of the sulfonamides and the partial explanation of their mode of action. Accordingly, it is desirable to review the more important findings upon which our present concepts of the action of sulfonamides are based.

Early in the study of the action of sulfanilamide it became evident that its effectiveness is not dependent upon a stimulation of the defense mechanisms of the body or on a simple germicidal action in the ordinary sense. Thus, the presence of a low concentration of sulfanilamide may have little effect for several hours on the rate of reproduction of microorganisms *in vitro*, although ultimately a bacteriostatic action is evidenced.

The effectiveness of sulfanilamide was shown to be dependent, within certain limits, upon the concentration of the drug, but other factors of importance have been demonstrated. Among these are the phase and strain of the bacterial species, the size of the inoculum, and particularly the composition of the culture medium. These facts led McIntosh and Whitby (1) in 1939 to suggest that the delay in onset of sulfonamide action might be due to "a slow combination or neutralization by the drug operating on some essential food substance or enzyme." Even earlier (1938), Lockwood (2) concluded from experiments with peptones that "the population curve in any medium is a resultant of the combined activities of peptone and sulfanilamide," and suggested that the drug might interfere

<sup>1</sup> This review is based on an earlier paper prepared while the author was a member of the staff of the Medical Research Division of Sharp & Dohme Inc., Glenolden, Pennsylvania and presented before the New Jersey section of the American Chemical Society and the A.A.A.S. Gibson Island Conference of July, 1944.



with the production or activity of certain enzymes. It was his thesis that the enzymic degradation of proteins accomplished by certain invasive organisms is prevented by sulfanilamide, but that the various lytic products, such as peptone, by supplying nutritive material, could abolish the effectiveness of the drug.

A major portion of the inhibitory action exerted by various peptones, and by extracts of yeasts, bacteria, and various tissues, can now be accounted for largely by their content of a specific substance, the action of which is antagonistic to the effect of sulfanilamide, and its various active derivatives, and upon which the life processes of many microorganisms appear normally to be dependent. Prominent among those workers whose findings justify this conclusion are the Englishmen, Stamp, Woods, Fildes, and McIlwain, and the Americans, Blanchard, MacLeod and Wood.

Stamp (3) showed in 1939 that heat-killed bacterial cells, as well as extracts of them, enabled homologous "organisms to grow in dilutions of sulfanilamide normally producing complete inhibition." He found that the activity of such extracts was not destroyed by heat, acids or alkalis and suggested that split products of protein might be responsible, although the possibility that the factor supplied might be "a substance of the nature of a coenzyme" was also given emphasis. Stamp's studies, however, did not appear adequately to exclude the possibility that his anti-sulfanilamide factor functioned simply as a stimulant of bacterial growth, rather than as a specific inhibitor of the action of sulfanilamide.

In 1940 Green (4) reported that an extract of *Brucella abortus* organisms greatly accelerated the rate of reproduction of homologous organisms in the presence of sulfanilamide. Addition of such bacterial extracts to cultures which appeared to have been sterilized by the previous addition of sulfanilamide caused a resumption of growth, while the bacteriostatic action of thionin was not antagonized by the extracts. However, Green was unable to demonstrate the presence of the anti-sulfanilamide factor in peptone or in extracts of other materials.

The studies of MacLeod (5), on the other hand, showed that a variety of peptones and tissue-extracts are capable of exerting marked anti-sulfonamide action. In certain tissues he found only small amounts of sulfonamide-inhibitor when the material was fresh, whereas after autolysis a relatively large amount was present, in human urine only a trace of activity was found prior to hydrolysis with acid. A possible clue to a mechanism of resistance to sulfonamides was uncovered by MacLeod, who noted that the bacterial production of sulfonamide-inhibitor was markedly increased, as resistance to sulfapyridine was developed in a strain of virulent pneumococci (type I).

In 1940 Woods and Fildes announced, in a preliminary report (6) which was expanded later by Woods (7), that *p-aminobenzoic acid* is enormously active as an anti-sulfonamide factor. A concentrate prepared from yeast was found to contain a factor resistant to destruction by heat, acid or alkali. The active substance was destroyed by nitrous acid and was diazotizable, its effectiveness, which was lost on acetylation or esterification, was regained following hydrolysis of such derivatives. These properties, together with measurements of solu-

bility and acid-dissociation, led logically to a trial of synthetic p-aminobenzoic acid. Although isolation of the naturally occurring inhibitor was not accomplished by Woods and Fildes, this was soon reported by Rubbo and Gillespie (8), who obtained from 30 kgm of wet yeast 2 mgm of a five-times recrystallized, benzoylated material with the proper melting point for p-benzoylamino benzoic acid, an identification which was not absolute. Blanchard (9), suspecting that much of the inhibitor in yeast is in a combined form, allowed fresh baker's yeast to autolyze with ethyl acetate prior to extraction, obtained the factor in pure form and identified it conclusively as p-aminobenzoic acid. He suggested that in nature the substance exists largely in a combined form in which the p amino group is blocked, perhaps in a manner resembling a peptide linkage.

In a paper accompanying that of Woods (7), Selbie (10) showed that the anti sulfonamide effect of p-aminobenzoic acid (*paba*) is not restricted to the test tube, since the chemotherapeutic action of sulfanilamide, in mice infected with a lethal strain of hemolytic streptococci, is also nullified by the administration of *paba*. The significance of the factor to the metabolism of certain microorganisms was first demonstrated by Rubbo and Gillespie (11), who found that with these organisms reproduction fails unless *paba* is present in their environment. However, as is evident from a number of investigations, the majority of the bacterial species susceptible to the action of sulfanilamide and its derivatives is not dependent upon external supplies of *paba*, since such organisms are able to synthesize adequate amounts of the material.

In 1940 Fildes (12), in whose department much of the work which led to our present concepts was done, discussed the significance of the data available at the time and suggested that *paba* should be looked upon as an "essential metabolite" with the utilization of which sulfanilamide and its derivatives interfere. Although for several reasons this term is not altogether satisfactory it has come into fairly general use. *Essential metabolites* may be defined as obligatory components of the normal sequence of metabolic reactions occurring in a living cell, they consist of two main types: *endogenous*, which are supplied by the synthetic activities of the organism, and *exogenous*, which an organism must obtain from its environment, although they are produced by the metabolic activity of other forms of life. Essential metabolites of the latter type are often referred to as "*essential growth factors*" and, of course, for those species requiring them, include those substances commonly referred to as vitamins. Under certain conditions essential metabolites may be synthesized slowly, but at a rate incompatible with optimal performance. Such a circumstance may enable an organism to survive under poor environmental conditions, but to thrive luxuriantly when the need for synthesis is obviated.

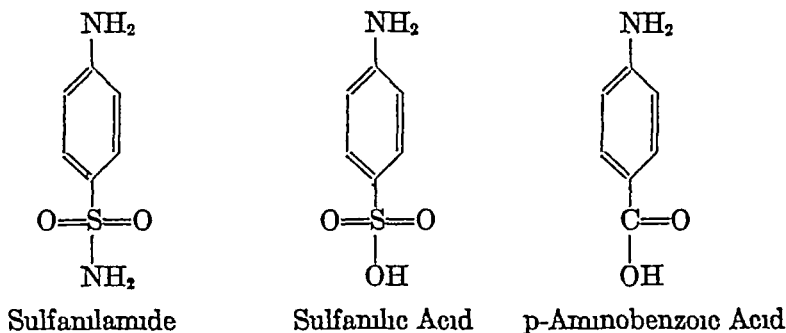
The obvious chemical similarity between the mutually antagonistic substances, sulfanilamide (p-aminobenzene-sulfonic acid amide) and *paba* (p-aminobenzene-carboxylic acid), suggested immediately that those components of the bacterial cell which normally combine with *paba* may also have an affinity for sulfanilamide. In admixture the two compounds would thus enter into competition for such components, and as a result two products would be formed, one presumably

essential to the course of a vital process, and the other largely or entirely inactive in that respect. Dependent on the ratio of the analog (sulfonamide) to the essential metabolite (paba), the physiological response would vary from growth and reproduction of a normal character to the complete obstruction of a reaction essential to life or reproduction. This view of the interference with the function of an essential metabolite, through the formation of a compound composed in part of a metabolite-like substance, is quite analogous to the combination between an enzyme and its substrate or a substrate-analog, that is, a compound in some respect chemically similar to the substrate. With many enzymic systems, the normal and the abnormal compound antagonize one another competitively and the kinetics of the reaction usually can be explained with mathematical precision.

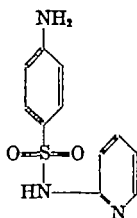
The term *competitive inhibitor* can be applied to the mutually antagonistic components of a system in which the rôle of either the metabolite or the anti-metabolite is reversibly and competitively interfered with by its antagonist, the relative amounts of the two substances in combination with the enzymic or other cellular components being dependent upon their relative affinities for them and upon their concentration-ratio.

A simple analogy may be drawn if we picture the natural substrate or metabolite as a key, so notched and grooved as to fit perfectly the mechanism of a lock which it operates smoothly and efficiently. The interfering substance, anti-metabolite, metabolite-analog, or enzyme-antagonist, may then be characterized as an imperfect key which, by virtue of its similar grooves, is able to enter the lock but is unable to operate its mechanism, although by its position in the lock it interferes with the entrance of the proper key.

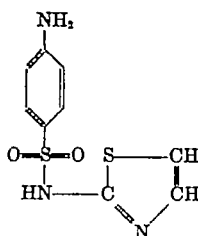
According to this view the more closely a sulfonamide resembles paba in those properties which enable it to react with some specific constituent of bacterial cells, without forming a functional complex, the more efficiently may it be expected to function as an anti-metabolite or blocking agent. At first glance it might appear that these requirements should be satisfied better by sulfanilic acid than by sulfanilamide, and certainly more closely by these compounds than



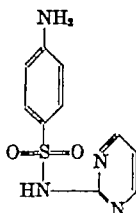
by the more active, but more complicated, heterocyclic derivatives of sulfanilamide, such as sulfapyridine, sulfathiazole, sulfadiazine and sulfamerazine. It has now been demonstrated, however, that those substances derived from sulfanilamide which are more effective chemotherapeutically than is sulfanilamide are also much more efficient in interfering with the action of paba, and no com-



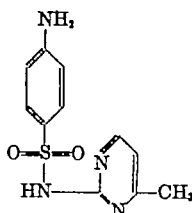
Sulfapyridine



Sulfathiazole



Sulfadiazine



Sulfamerazine

pound has been found, which appears to behave like sulfanilamide, that is not effectively inhibited by paba.<sup>2</sup>

Extensive study of the physico-chemical properties of compounds antagonistic to paba has led, during the last two or three years, to marked advances in our concepts of those structural characteristics which enable compounds to compete effectively with paba for the components of bacteria which utilize that substance. At one time it was considered that the greater activity of some derivatives of sulfanilamide containing heterocyclic ring-substituents in the N<sup>1</sup> position, might be due to the ability of such derivatives to inhibit other systems, in addition to those involving paba. The possibility of a "double-barreled" action will not be thought unreasonable, when it is considered that nicotinic acid, an important essential metabolite, contains within its molecule the pyridine ring found also in sulfapyridine. Further, in thiamine there are found the thiazole and the pyrimidine rings. These heterocyclic rings find their counterparts in sulfathiazole, on the one hand, and in the pyrimidine derivatives, sulfadiazine, sulfamerazine and sulfamethazine on the other. Despite the interesting features of this theory, it now appears well established that although certain N<sup>1</sup>-heterocyclic groups may indeed confer demonstrable accessory inhibitory characteristics upon sulfanilamide (West and Coburn, 13, Dorfman and Koser, 14), these do not contribute significantly to the chemotherapeutic activity of the compounds in the concentra-

<sup>2</sup> The properties of Marfanil  $\text{H}_2\text{N}-\text{CH}_2-\text{C}_6\text{H}_4-\text{SO}_2-\text{NH}_2$  differ sufficiently from those of sulfanilamide as to suggest that different mechanisms are involved in the bacteriostatic actions of the two compounds.

tions found effective (15, 16) Moreover, several very active sulfonamides are known which contain heterocyclic rings, the occurrence of which in microorganisms is believed to be unlikely Clearly, the potentiating effect of such N<sup>1</sup>-substitutions on the activity of sulfanilamide cannot reasonably be attributed to contributory effects on systems involving essential metabolites other than paba

Several workers have considered the bacteriostatic activity of the sulfonamides to be related to the ability of these compounds to dissociate as acids Bell and Roblin (17) suggested that the more negative is the  $\text{—SO}_2\text{—}$  group of a sulfonamide, the more closely does the group resemble the  $\text{—CO}_2\text{—}$  group of paba at pH 7, at which pH the metabolite is over 99.9 per cent in the anionic form and the  $\text{—CO}_2\text{—}$  group possesses a strongly negative charge These workers showed that in a buffered medium of pH 7, *in vitro*, the bacteriostatic activity of a group of sulfonamides increased as the pK<sub>a</sub> increased, until maximal activity was attained at pK<sub>a</sub> values ranging between 6 and 8.5 Within their series of compounds bacteriostatic activity fell off steadily with further increase in the values for pK<sub>a</sub> Since the negativity of the  $\text{—SO}_2\text{—}$  group is greatly increased by the loss of a proton from the adjacent nitrogen atom, the ionic form of any sulfonamide, according to this theory, would be much more active than the molecular form, and hence bacteriostatic power, as measured at pH 7, would increase with acid strength until complete ionization was attained Compounds having pK<sub>a</sub> values of about 6 or higher would be essentially completely ionized at pH levels of 7 or above The decrease in bacteriostatic activity observed with further increases in the values for pK<sub>a</sub> was considered by Bell and Roblin to be related to a second influence, namely, that acid strength is proportional to the electron-attracting power of the N<sup>1</sup>-substituent As this power increases beyond values sufficient to permit complete ionization at pH 7, the substituent group would attract electrons away from the  $\text{—SO}_2\text{—}$  group, thus decreasing its negativity, and hence the bacteriostatic activity of the compound

Although the theory of Bell and Roblin, as tested by their experimental procedures, appears to have certain limitations, in that it does not appear entirely satisfactorily to account for the observed activity of non-acidic compounds, their concepts unquestionably mark a real milestone in progress along the road of correlation between chemical constitution, physical properties, and pharmacological action

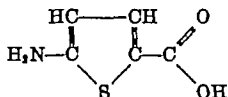
A further extension of this concept of the relation of structure to activity in the group of compounds which antagonize paba is to be found in the recent papers of Kumler and his associates (18, 19, 20) Although their concepts cannot be dealt with in detail, these workers place emphasis on the relation of resonance to the negative character of the  $\text{—SO}_2\text{—}$  group The theory is said to account satisfactorily for the activity exerted by certain compounds that are incapable of ionizing as acids, and which form exceptions to the theory of Bell and Roblin Kumler and his associates also suggest that the neutral molecules of ionizable sulfonamides may more efficiently reach the site of action, where "the ion then interferes with essential metabolic processes resulting in bacteriostasis" The conclusions reached by Kumler and his associates cannot be taken as final, since they have been challenged and disputed in recent articles by Bell, Bone and

Roblin (21) and by Bordwell and Klotz (22) Mention should also be made of the paper by Klotz (23) in which activity is related to acidity and to the effect of mass action upon an enzyme system

It is known that in the blocking of the utilization of paba by one organism, *in vitro*, one derivative of sulfanilamide may prove definitely superior to another, while with some other bacterial species the relative merits of the two compounds may be quite reversed This does not signify necessarily that our basic concepts of competitive inhibition are incorrect, but rather that our understanding of the various complicated processes involved, such as penetration, adsorption and absorption, is far from complete For example, it is not unreasonable to consider that the relative insensitiveness of the tubercle bacillus to the various ionizable sulfonamides may be attributable to the effect of the lipoidal constituents of this microorganism on the penetration of the drug

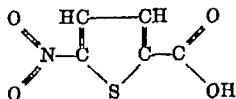
The important fact has been mentioned previously that several compounds related chemically to paba which have non-acidic properties quite different from those of sulfanilamide, e g, certain sulfones, ammobenzils, aminoacetophenone, N methyl compounds, and the like, possess bacteriostatic properties which are blocked by paba Additional evidence for the concept of bacteriostasis through the antagonism of paba has been advanced by the studies of Johnson, Green and Pauli (24), who found that ring-substituted, and other derivatives of p-aminobenzoic acid are anti-bacterial *in vitro* and that their bacteriostatic activity is blocked by the addition of paba, none, however, is as active as sulfanilamide Particularly interesting were their studies of various new compounds in which the thiophene ring was substituted for the benzene ring of paba These analogs are comparable to a modification of thiamine (to be discussed later) in which its thiazole ring is replaced with the pyridine ring, yielding a compound (pyrithiamine) which effectively antagonizes the utilization of thiamine In one case a  $-\text{CH}=\text{CH}-$  is replaced with  $-\text{S}-$ , in the other the change is in the reverse direction

The thiophene analog of paba, 2-amino-5-carboxythiophene (aminothiophenic acid), is too unstable to test directly, and only derivatives or related compounds

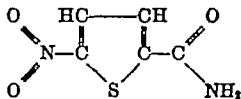


2-amino-5-carboxythiophene

have been studied Johnson et al. (24) found, however, that the bacteriostatic action of the corresponding nitro compound and its amide is blocked by paba and

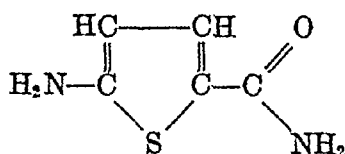


2-nitro-5-carboxythiophene



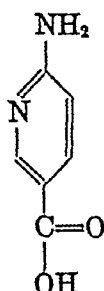
Amide of 2-nitro-5-carboxythiophene

compares favorably with the activity of the most active sulfonamides, at least on *Streptococcus hemolyticus*, *in vitro*. Since the reduction product derived from the bacteriostatically active amide of 2-nitro-5-carboxythiophene, namely, the amide of 2-amino-5-carboxythiophene, is inexplicably inactive, Johnson and his co-



Amide of 2-amino-5-carboxythiophene

workers suggest that some reduction product of the nitro group, which more closely resembles an aromatic amino group, may be responsible for the bacteriostatic activity of the nitro compound. Although replacement of the benzene ring of paba with the pyridine ring yielded a compound with considerable activity



2-amino-5-carboxypyridine

against *E. coli* and streptococci, it was, for reasons at present unknown, unable to influence the metabolism of pneumococci. Analogs derived from thiazole or from furane were apparently too remotely related to paba to exert activity.

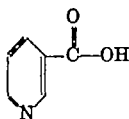
Miller (25) has also tested certain thiophene derivatives related to paba, with results in essential agreement with those of Johnson et al. (24). Since Auhagen (26), using a species of *Lactobacillus*, reported that p-aminobenzoyl-glutamic acid possesses considerably greater antisulfonamide activity than paba, this compound was also tested by Miller. However, with *E. coli*, under the conditions of the experiments, its activity appeared to be less than that of paba. Williams (27) also found this compound to be much less active than paba, when tested on *E. coli*, *S. hemolyticus*, *D. pneumoniae* I, and *Cl. acetobutylicum*.

The possibility that Woods' discovery of the antagonistic effect of paba on the action of sulfanilamide might have general significance was immediately apparent to several workers. In 1940 Fildes (12) suggested the possibility that new chemotherapeutic agents might be developed through the preparation of various structural modifications of compounds known to function as essential metabolites. In this manner it was hoped to find substances substantially incapable of producing the physiological effect of an essential metabolite, but possessing high affinity for those enzymes, or other components of cells, with which the metabolite reacts. Although as yet none of the analogs concerning which re-

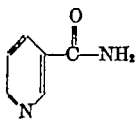
ports are available has given rise to a new series of practical chemotherapeutic agents, there is as yet no apparent reason why, in the more or less blind groping which led to the discovery of "Prontosil," science should have stumbled upon the only metabolite of microorganisms the function of which can be effectively antagonized within the body of an infected host. In this connection there is only a very remote possibility that antibiotic agents, such as penicillin, function as antagonists of essential metabolites, probably, however, because of the minute quantities which are required, there occurs some more subtle interference with enzymic systems vital to the continued function of a variety of microorganisms.

Quite logically the first attack on the problem of expanding the concept of metabolite-antagonism, was directed toward structurally identified metabolites which, although essential to the metabolism of microorganisms, were known to be required in very small amounts. Particularly active and effective in such studies have been McIlwain in England, and Woolley and Snell in this country.

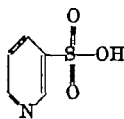
*Nicotinic acid analogs* In 1940 McIlwain (28) described studies with pyridine-3-sulfonic acid and its amide, showing that these compounds effectively inhibit various bacterial species for which nicotinic acid, nicotinamide, or certain complexes of them, are essential growth factors.



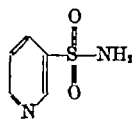
Nicotinic Acid



Nicotinamide



Pyridine-3-sulfonic acid



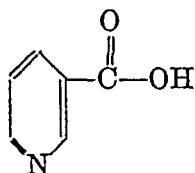
Pyridine-3-sulfonamide

In contrast to the antagonism between paba and the sulfonamides, it appears that these nicotinic analogs are unable to interfere with the metabolism of those bacterial species which obtain their supply of derivatives of nicotinic acid by synthesis. Conceivably this inactivity may reflect a failure of the analogs to permeate the bacterial cells and to attain a sufficient concentration in that portion of the cytoplasm where nicotinic compounds or their complexes are synthesized. According to this view, effective inhibition by the analogs of those bacteria requiring an external source of nicotinic acid, or its amide, would appear to be dependent on an interference with the absorption of the growth factor by the organism. Whatever the mechanism, it is clear that other factors operate in addition to that of a simple competition between the metabolite and its antagonist, based on the relative numbers of the molecules of each and their relative affinity for the cellular component with which they react. McIlwain found from his studies of the effect of pyridine-sulfonic acid and its amide on the growth of staphylococci, and on species of *Proteus*, that there is a marked lack of equivalence of nicotinic acid, nicotinamide, and its derivative, diphosphopyridine nucleotide. In some cases, for example, inhibition with pyridine-3-sulfonic acid was more intense than with its amide and it was tentatively suggested that the

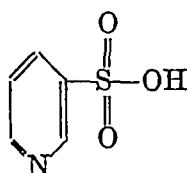


sulfonic acid may affect most powerfully a reaction at some unknown stage higher than cozymase

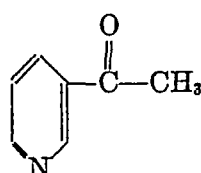
Since several animal, as well as bacterial, species require an exogenous supply of nicotinic acid or its amide, it would be of interest to consider whether the administration of pyridine-3-sulfonic acid to animals interferes with the utilization of the vitamin Woolley et al (29) in 1938 examined a series of substances for nicotinic acid-like activity in dogs with experimentally induced black tongue, and found that although pyridine-3-sulfonic acid and  $\beta$ -acetyl-pyridine produced none of the actions of nicotinic acid as a vitamin, they did exert a markedly toxic



Nicotinic acid



Pyridine-3-sulfonic acid

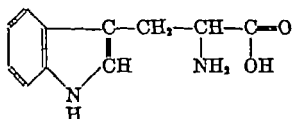
 $\beta$ -acetylpyridine

or even lethal effect in the deficient animals Such effects, significantly enough, were not seen when either of the compounds was given, in similar doses, to dogs ingesting adequate amounts of nicotinic acid In the light of present knowledge, these results strongly suggest that the two synthetic derivatives of pyridine inhibited the proper utilization of nicotinic acid by dogs whose stores of the vitamin were severely depleted and that rapidly toxic effects were thus produced In healthy animals, however, the supply of nicotinic acid derivatives was adequate to compete with and thus to prevent the blocking effects of the analogs It is possible that if sufficiently large amounts of the compounds were given, interference with the nicotinic acid metabolism of the normal dog could be accomplished A reinvestigation of this subject might prove profitable

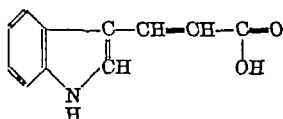
Woolley and White (30) showed that in the mouse, which appears to fulfill its requirements for nicotinic acid derivatives by synthesis, pyridine-3-sulfonic acid, in the dosage used, produces no evidence of deleterious effects These workers pointed out that this failure may be due to a mechanism similar to that responsible for the lack of bacteriostatic effect on microorganisms which synthesize their own supply of nicotinic derivatives A very recent report (31) indicates, however, that nicotinic acid deficiency can be produced in the mouse with  $\beta$ -acetylpyridine, so that the explanation for the failure with pyridine-3-sulfonic acid is not readily apparent Possibly, however, differences in the penetration of cells by  $\beta$ -acetylpyridine and pyridine-3-sulfonic acid are responsible for the difference in their activity, it will be noted that the former is non-acidic while the latter is an ionizable acid

*Amino acid analogs and pyridoxine* In 1941 McIlwain (32) found that certain aliphatic  $\alpha$ -aminosulfonic acids inhibit a number of microorganisms by virtue of their structural similarity to various  $\alpha$ -aminocarboxylic acids It is worth noting that this type of antagonism is unspecific, since the effect of an  $\alpha$ -aminosulfonic acid is removed by  $\alpha$ -aminocarboxylic acids other than that which is strictly analogous to the inhibitor McIlwain pointed out that the "Production

of a compound capable of interfering with a particular metabolite evidently demands consideration of properties of which structural formulae give only approximate indications," a statement the truth of which is becoming increasingly evident, particularly, of course, from the study of the sulfonamides, as has been discussed previously



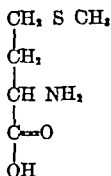
Tryptophane



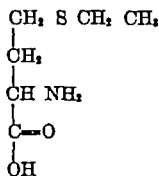
Indoleacrylic acid

Fildes (33) showed in 1941 that a compound resembling tryptophane, indole acrylic acid, in a concentration of M/8000 to M/1000, will block the growth of *E. coli* on an ammonium lactate medium, this effect is reversed by tryptophane, but there is no quantitative relationship between the effect of the two compounds. From this fact and because the addition of only a trace of tryptophane causes growth in spite of the inhibitor, Fildes suggested that indoleacrylic acid interferes with the synthesis of tryptophane, rather than with its utilization.

It is interesting to note that a phenomenon probably to be attributed to metabolite-antagonism was recorded in 1938 by Dyer (34), in duVigneaud's laboratory. In feeding experiments, ethionine, the S-ethyl homolog of methionine, was unable to support the growth of rats on a diet deficient in cystine and



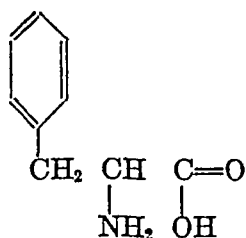
Methionine



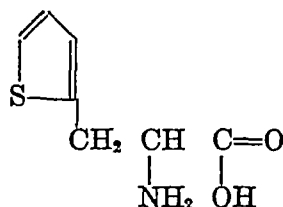
Ethionine

methionine, and also caused certain toxic manifestations. Animals given the homolog showed a loss in appetite, lost weight more rapidly than rats on the basal diet alone, and died after short periods of supplemental feeding. When methionine and ethionine were administered together, the loss of appetite was less marked and the animals usually gained weight, although not as rapidly as when ethionine was excluded from the diet. It now appears reasonable to explain these results on the basis of competitive inhibition of the utilization of methionine by its higher homolog. Conceivably the compound interferes with the ability of methionine to serve as a donor of methyl groups although other functions of the amino acid may be affected as well. Harris and Kohn (35) have observed that ethionine limits bacterial growth, apparently by interfering with the bacterial metabolism of methionine.

The metabolism of phenylalanine, an amino acid essential for growth in the rat, has been shown by duVigneaud (36) to be affected deleteriously by thienylalanine, a compound in which the benzene ring of phenylalanine is replaced by



Phenylalanine

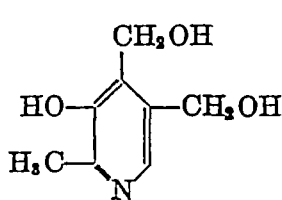


Thienylalanine

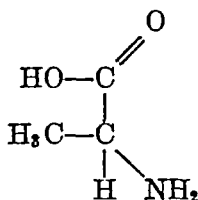
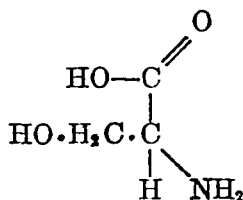
the thiophene ring. It was found that the growth of rats is inhibited by the inclusion of this isostere in the diet, an effect competitively antagonized by the naturally occurring amino acid. The growth of yeast also served to demonstrate the competitive antagonism between phenylalanine and the isostere.

There are several examples of metabolite-antagonism involving amino acids and other growth factors which have not yet been completely explored. Snell and Guirard (37) showed that under certain circumstances the requirement of *Streptococcus lactis R* for pyridoxine can be completely satisfied by  $\alpha$ -alanine. Excessive amounts of glycine, however, and to a lesser extent of serine or of threonine, inhibit the growth of this organism, these inhibitions can be removed, within limits, by pyridoxine, and even more effectively by alanine, but not by other vitamins or amino acids. Snell and Guirard comment on the fact that both glycine and serine can produce toxic effects in animals. At Snell's suggestion, Fishman and Artom, who discovered the toxic effect of dl-serine when administered to rats by stomach tube (38, 39), studied the effect of pyridoxine on the toxic effect of the amino acid (40) and found that, of the various vitamins tested, it is the most effective preventive of the toxic manifestations caused by serine.

Examination of the structural relationship between pyridoxine and alanine or serine shows a most interesting similarity, as has been pointed out by Snell



Pyridoxine

 $\alpha$ -Alanine

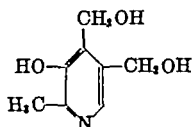
Serine

Despite the structural similarities the mechanism responsible for the antagonism between pyridoxine and alanine or serine is not known. Speculation is probably unwise at present, in view of the complications introduced by Snell's brilliant elucidation of the chemical nature of pseudopyridoxine (41), which has now been resolved into two compounds, pyridoxamine and pyridoxal, both closely related

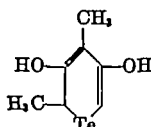
to pyridoxine and both now available in synthetic form as a result of the work of Harris, Heyl and Folkers (42)

Study in animals of various antagonists of the essential amino acids, and of other compounds important for structural as well as purely functional reasons, should prove to be most helpful in uncovering much that is now hidden. As possible chemotherapeutic agents, however, such compounds offer less promise, since the relatively large requirement of the animal body for these nutrients greatly reduces the possibility of their analogs having practical utility.

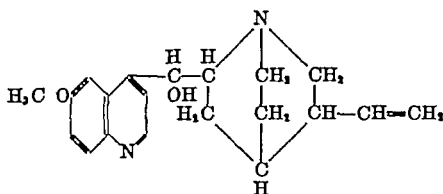
Another phenomenon possibly involving the action of pyridoxine on bacteria was postulated by Gulland and Farrar (43), who found that certain very toxic compounds resembling pyridoxine in structure, but containing tellurium in place of the nitrogen of the pyridine ring, were effective bactericidal agents, *in vitro*, against *E. coli*, *B. typhosus*, *Staph. aureus*, and *Strep. hemolyticus*. The structural formulae of pyridoxine and of their most active compound of tellurium are



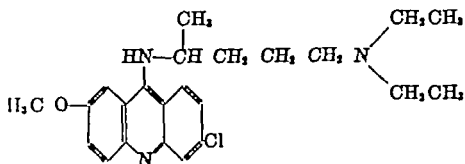
Pyridoxine

2,4-dimethyl-cyclotelluropentane  
3,5-dione

It is unfortunate that the authors were unable to test experimentally their hypothesis, which appears reasonable, that the tellurium-containing compounds interfere with the metabolism of pyridoxine.



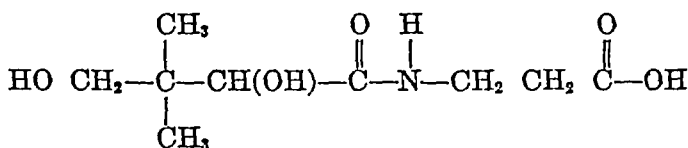
Quinine



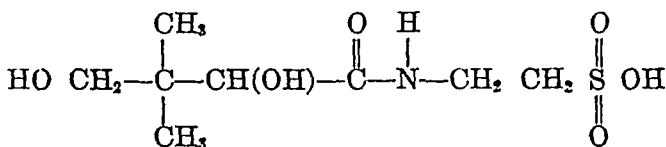
Atabrine

A most striking observation made recently by Seeler (44) suggests a relation between the action of quinine or atabrine and that of pyridoxine. It was shown that the therapeutic effect of these antimalarials, given by mouth to ducks infected with *Plasmodium lophurae*, is antagonized markedly when massive doses of pyridoxine are administered orally or subcutaneously. Seeler suggests that the vitamin, or a compound of similar structure, is essential for the life of the parasite and that quinine and atabrine act by competing with a pyridoxine-like compound for a position in some enzyme system of the parasite. As Seeler points out, a note of caution concerning this interpretation is offered by the results of Silverman and Evans (45), who found that on *E. coli*, *in vitro*, pyridoxine, under their experimental conditions, failed to affect the action of atabrine. Whether the results prove to be due to an inhibition of a truly competitive nature, or are less directly obtained, they are almost certainly of importance and could lead to improvements over drugs now available for the treatment of malaria.

**Pantothenic acid analogs** Extensive studies have been carried out with various pantothenic acid-like compounds, particularly with the sulfonic acid analog, variously termed pantoyltaurine and thiopanic acid, and with the corresponding amide, pantoyltauramide.



Pantothenic acid



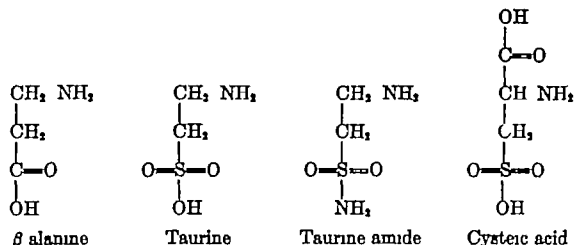
Pantoyltaurine

Snell (46) showed in 1941 that pantoyltaurine markedly inhibits the growth of *Lactobacillus arabinosus* and, in large amounts, the growth of a strain of yeast, this inhibition is reversed by the addition of calcium pantothenate, so that a truly competitive inhibition appears to occur. Essentially all the activity of racemic pantoyltaurine was shown to reside in the optical isomer that corresponds to the naturally occurring isomer of pantothenic acid. Snell found that the sensitivity to pantoyltaurine of various organisms which require pantothenic acid varies markedly. Various bacterial species which do not require pantothenic acid (*E. coli*, *Staph. aureus*, *Shigella paradyseuterae*, *Brucella abortus*) were not inhibited by analogs of pantothenic acid, a situation resembling that encountered with the analogs of nicotinic acid.

Since certain organisms require an exogenous supply of  $\beta$ -alanine, but not of the pantoic acid moiety of pantothenic acid, it is of interest to note that the

growth of *Saccharomyces cerevisiae* induced by  $\beta$ -alanine is not antagonized by pantoyltaurine. This observation might be interpreted as meaning that  $\beta$ -alanine in this case served otherwise than as a precursor of pantothenic acid, however, it appears more reasonable to suggest that pantoyltaurine fails to inhibit the growth of this strain of yeast for the same reasons it fails in other cases where pantothenic acid is synthesized by an organism.

Taurine, the sulfonic analog of  $\beta$ -alanine, was found by Snell (47) to be without influence on the utilization of the amino acid, and McIlwain (32) showed that taurine, taurine amide and cysteic acid were not inhibitory to any of the various organisms studied.



Hartelius (48) observed, however, that although  $\beta$  aminobutyric acid does not influence the effect of pantothenic acid on the respiration of yeast, it does inhibit the activity of  $\beta$  alanine.

The first of McIlwain's several studies of pantothenic acid derivatives appeared in 1942 (49). Eight compounds were examined, but of these only pantoyltaurine and its amide proved to be markedly inhibitory to bacterial growth. On a strain of *Streptococcus hemolyticus*, in the presence of  $10^{-7}M$  pantothenate, pantoyltaurine and its amide (and to a lesser extent, homopantoyltaurine) markedly inhibited growth—an inhibition which was reversed by the addition of extra pantothenate.

In this and in other studies (50, 51), McIlwain has recommended the use of the convenient term, *antibacterial index*, which represents the minimal value of  $C_I/C_M$ , that is, the ratio of the concentration of inhibitor ( $C_I$ ) just sufficient to prevent the growth of an organism, to the concentration of metabolite ( $C_M$ ) present. Obviously, the smaller the antibacterial index the more effective is the compound as an inhibitor. Under the conditions described, and using *Streptococcus hemolyticus*, the following antibacterial indices were found: homopantoyltaurine—20,000, pantoyltauramide—2,000, pantoyltaurine—500. For pneumococci (types I, II or III) the antibacterial indices were: pantoyltauramide—10,000, pantoyltaurine—1,000. The pantoyltaurine used was racemic, so that the true activity is approximately twice that indicated in the above figures.

Bacteria which synthesize their own supply of pantothenate, such as *E. coli* and *Proteus vulgaris*, are not inhibited by these analogs of pantothenic acid. This failure cannot be attributed, however, to the synthesis of unduly large

amounts of pantothenate by the microorganisms, the mechanism of the resistance is at present unknown. One can only hypothesize that the synthesis may occur at such a locus within the cell that a sufficient concentration of the analog cannot be attained at the site of utilization, or that the metabolite synthesized is so efficiently used at the site of synthesis, or is used in such a modified form, that the analog cannot compete in the system involved. An even more difficult situation to explain is found in the case of *Proteus morganii*, an organism which requires exogenous supplies of pantothenate, but which fails to be inhibited by an excess of pantoyltaurine.

Calculation of the antibacterial indices of pantoyltaurine, pyridine-3-sulfonamide and sulfanilamide shows that, under the conditions of the determinations of McIlwain, hemolytic streptococci are most effectively inhibited *in vitro* by pantoyltaurine.

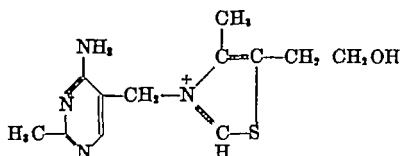
The findings described indicate clearly that pantoyltaurine might be expected to function as a chemotherapeutic agent in streptococcal and pneumococcal infections of animals. Accordingly, McIlwain and Hawking (52) studied the effect of the compound in mice infected with hemolytic streptococci. The substance, which was shown to be extremely well-tolerated by this species, was given repeatedly by a parenteral route, at intervals of about 8 hours, each dose was about 0.1 gram, equivalent to a daily dosage of about 12 grams per kilogram of body weight. The failure of the compound to protect the mice against streptococcal infection was accounted for by the very high levels of pantothenate in the blood of this species. Thus, when the experiment was repeated in rats, in which the level of pantothenic acid in the blood is lower than in mice, a high degree of protection was observed. During each day about 10 grams per kilogram were given subcutaneously, at intervals of about six hours, for a period of four days. The effective chemotherapeutic action exerted by the analog, under these circumstances, was abolished by the simultaneous administration of one-fiftieth the amount of pantothenate. The analog thus appears to interfere competitively with the utilization of pantothenic acid by the bacteria invading the tissues of the animals.

It is evident from the size and frequency of the dosage required to protect the infected rats that pantoyltaurine is without utility as a practical chemotherapeutic agent for the treatment of streptococcal infections. This failure may be attributed to the high concentration of the vitamin in mammalian tissues and to the extreme difficulty of maintaining an adequate concentration of the drug in the blood and other tissues. It is not inconceivable, however, that other modifications of the structure of pantothenic acid may be devised which would enable such compounds to compete effectively with pantothenic acid, and, at the same time to exhibit a more favorable rate of renal elimination, since that would appear to be a prominent cause of difficulty, and perhaps to affect organisms other than those dependent upon their environment for a supply of pantothenic acid. A compound with such characteristics might function as an effective chemotherapeutic agent, in addition to the sulfonamides and penicillin, for the treatment of streptococcal, pneumococcal, and other infections.

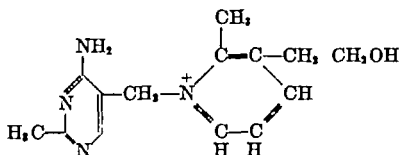
Barnett has described (53) the preparation of a group of substances closely related to pantooyltaurine, but none of these analogs is more active than pantooyltaurine as an inhibitor of *Lactobacillus arabinosus*, *in vitro*, or of *Streptococcus hemolyticus*, *in vivo*

The levels of pantothenic acid and of its analog which were encountered by McIlwain in the blood of mice, strongly indicate that antagonism of the utilization of pantothenic acid in this species (or in the rat) would require fantastically large amounts of pantooyltaurine. Although a preliminary study conducted cooperatively by groups in California and Texas (54) indicated that feeding relatively large amounts of the analog to mice produced signs of pantothenic acid deficiency, this is now known almost certainly to have been artefactual (55, 56)

**Thiamine analog** One of the most interesting substances which has been studied in connection with the blocking of essential metabolites is pyriethamine, a compound in which the substituted thiazole ring of thiamine is replaced with a



Thiamine



Pyriethamine

similarly substituted pyridine ring. It might be anticipated that this compound would have physicochemical properties closely similar to those of thiamine, since the work of Erlenmeyer (57) had indicated the close similarity of ring systems in which an  $-S-$  is replaced by  $-CH=CH-$ , or *vice versa*. Robbins (58) found that the growth of certain fungi which are dependent upon exogenous supplies of thiamine is markedly inhibited by the addition of this compound to the culture media.

Woolley and White have investigated the effect of pyriethamine on mice (59) and as an inhibitor of the growth of various species of bacteria, yeasts and fungi (60, 61). Observations with microorganisms resembled those made with the analogs of nicotinic acid and of pantothenic acid, in that inhibition was found to occur only with those organisms which require for their growth an exogenous supply of the analogous vitamin. Organisms which synthesize thiamine are not



inhibited, even by large amounts of pyrithiamine, and the mechanism of resistance cannot be attributed to the synthesis of increased amounts of the corresponding vitamin. It would be interesting to determine, however, whether any pyrithiamine-sensitive organism could be "trained" to produce protective amounts of thiamine by culturing such strains in media containing progressively larger amounts of the analog. If such were the case the situation would be analogous to that which obtains in certain bacterial species that produce progressively larger amounts of p-aminobenzoic acid with continued exposure to progressively higher concentrations of a sulfonamide (62, 63, 64). On the other hand, under such circumstances, some bacterial species develop resistance to sulfonamides without any demonstrable increase in their production of paba (65). Woolley and White (60) showed that the resistance of bacterial species *initially* insensitive to pyrithiamine is not attributable to the production of large amounts of the vitamin, and no evidence of the production of any other inhibitor could be obtained. In a more recent communication Woolley (61) demonstrated that a yeast, *Endomyces vernalis*, could be made pyrithiamine-fast by transferring it 30 times in a thiamine-free medium containing 25 times the amount of pyrithiamine necessary to inhibit the growth of the parent strain one-half maximally. The new strain still required, as a growth factor, either thiamine or that portion of its molecule derived from pyrimidine. The resistance of the new strain was accounted for, at least in part, by a system that arose which was able to split the thiamine-analog into its pyrimidine and pyridine portions, the former apparently served the organism as a precursor for the synthesis of thiamine, which then blocked the effect of the intact analog remaining. It is worth noting that the pyridine moiety of pyrithiamine had no effect on the utilization of the thiazole portion of thiamine, when organisms were used that synthesize thiamine from its thiazole-fragment, only intact pyrithiamine was effective, a situation analogous to the failure of taurine to inhibit the utilization of  $\beta$ -alanine.

Wyss (66) determined the antibacterial indices of pyrithiamine with staphylococci and with *E. coli*. For the former, the index was about 700, for the latter, about 20,000. These results differ appreciably from those of Woolley and White (60), who with their strain of *E. coli*, obtained an index of more than 2,000,000, and an index of about 2,000 with *Staphylococcus aureus*.

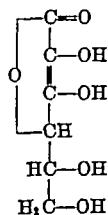
Woolley and White (59) have also studied the effect of pyrithiamine administration on weanling mice fed a highly purified diet. In this species the removal of thiamine from the diet causes only a gradual development of a deficiency syndrome, characterized by a progressive loss of appetite and loss of weight, with eventual death. However, with the exception of anorexia, the administration of adequate amounts of pyrithiamine caused the development of signs attributable to a severe thiamine deficiency. Within a period of 5 to 12 days, if sufficient pyrithiamine were given, and depending upon the ratio of it to the thiamine ingested, Woolley and White noted progressively inactivity, tremors, convulsions, spasticity, extreme weakness, and finally death within about 3 days from the first appearance of the signs of thiamine deficiency. It was calculated

that about 40 molecules of pyrithiamine would nullify the effect of 1 molecule of thiamine. Some of the data showing cumulative effects indicate, however, that the ratio is even more favorable to antagonism, apparently not exceeding 10 to 1. It is obvious that pyrithiamine is an extremely active antagonist of thiamine.

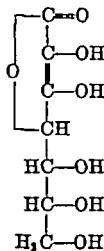
Unlike pantooyltaurine, pyrithiamine is apparently extremely slowly eliminated by the mouse, since delayed results were readily produced. For example, mice given 12 mgm daily for 3 days, and then continued, without pyrithiamine, on a daily dosage of 2 micrograms of thiamine grew normally for 6 days, but on the seventh day began to lose weight precipitously and to show the characteristic signs previously described. A single dose of 0.5 mgm of thiamine on the ninth day produced an apparent cure within 24 hours. Cumulative effects were demonstrated by administering very small daily doses (20 micrograms of pyrithiamine with 2 micrograms of thiamine) after two and a half weeks without evidence of thiamine deficiency, the characteristic changes developed.

Were it not for the extremely toxic effect of pyrithiamine on the animal it might well have proved useful as a chemotherapeutic agent, so marked is its effect on bacteria dependent on external supplies of thiamine. However, all data indicate that the vital importance of thiamine and the minute amounts of the vitamin needed by both bacteria and animals completely prevent the utilization of the analog in the chemotherapy of infectious disease. As a nutritional tool it should continue to prove valuable.

*Ascorbic acid analog* Particularly interesting is the work of Woolley and Krampitz (67) with glucoascorbic acid. Although at present it cannot be stated positively that the effect of this compound is attributable to metabolite-antago-



Ascorbic acid

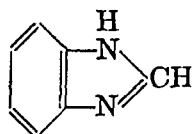


Glucoascorbic acid

nism, such an explanation would appear logical. When this compound was fed to mice at a 10 per cent level in a highly purified diet, there developed a syndrome characterized by failure in growth, diarrhea, subdermal hemorrhages, and death within 2 weeks. At a dietary level of 5 per cent the picture was similar, but death was either delayed or recovery occurred in spite of the continued ingestion of the compound. Such recovery was interpreted as signifying increased synthesis of the metabolite, with the function of which glucoascorbic acid interfered. In those animals which survived more than 2 weeks there developed an alopecia.

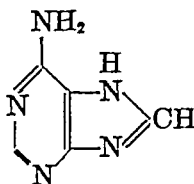
of the head and back that affected only those areas which did not become hairless as the result of a deficiency of inositol or of pantothenic acid. Two other interesting features were seen concerning the syndrome precipitated by this interesting compound: one, in mice it was not due to a simple antagonism of the utilization of ascorbic acid, since simultaneous feeding or injection of the vitamin did not prevent the development of the condition; two, the syndrome was prevented by the inclusion in the diet of various plant materials (powdered grass juice was effective at a minimal level of 10 per cent). The workers suggest that "certain plant substances contain a compound related to ascorbic acid in the normal course of its utilization, and that this substance is effective in overcoming the block to ascorbic acid metabolism caused by glucoascorbic acid." It would appear that through the use of glucoascorbic acid Woolley and Krampitz have discovered a clue to a new substance which is related to ascorbic acid and probably is a product of its metabolism. The close relationship of this new metabolite to vitamin C is also shown by the subsequent demonstration (68) in the guinea pig, a species dependent upon an exogenous supply of the preformed vitamin, that the deficiency syndrome characteristic of glucoascorbic acid is completely prevented by the simultaneous administration of ascorbic acid.

*Analogs of purines and pyrimidines* In surveying various compounds which might lend themselves to the synthesis of possible blockers of biotin metabolism, Woolley (69) examined the effect of benzimidazole on the growth of various organisms.

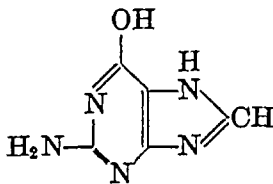


Benzimidazole

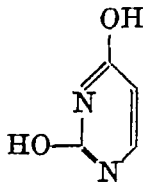
It was found that the toxic effect of this compound on a strain of *Saccharomyces cerevisiae* could be overcome by adenine or guanine, and the ratio of the amount of inhibitor to the amount of adenine required for reversal was about the same with high as with low doses of the drug. Hypoxanthine, xanthine, yeast adenylic acid and uracil were not antagonistic to benzimidazole.



Adenine



Guanine



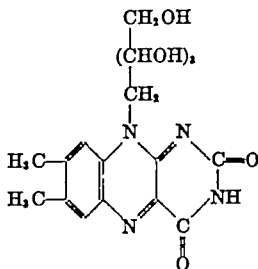
Uracil

Curiously, a compound even more closely related to adenine, namely, 4-amino-benzimidazole, was no more active than the unsubstituted compound. A remarkable observation, however, was that uracil, although inactive in reversing the effect of benzimidazole on the yeast, was the most active of all the compounds

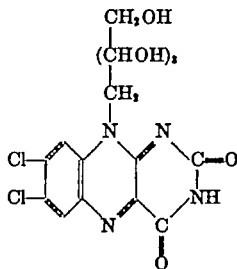
in causing an incomplete reversal of the effect of the inhibitor on *Streptococcus lactis* R. Also of interest was the finding that in mice the anesthetic effect of benzimidazole (10 mgm. intraperitoneally) was only very slightly modified by the administration of adenine sulfate (15 or 30 mgm.)

Presumably the effects described by Woolley (69) may be attributed to a competitive antagonism between benzimidazole and the naturally occurring compounds capable of reversing the growth inhibiting effects of the substance. Although a relationship between the structures of the purines, adenine and guanine, and that of benzimidazole can be visualized by exchanging the pyrimidine ring for the benzene ring, and *vice versa*, it is more difficult to interpret the effect of the simple pyrimidine, uracil. In this connection Snell (70) has suggested that the growth factor-effect of uracil may not have been entirely separated from the inhibitor reversal effect, since uracil is very effective in increasing the rate and extent of growth of the organism involved, and this substance was not included in the medium. Conceivably, if uracil were present in excess, adenine might have blocked the effect of imidazole on this bacterial species, as it did for yeast. Further study in this field may prove profitable.

**Riboflavin analogs** A preliminary report by Kuhn and his co-workers (71) describes the effect of a compound in which the two methyl groups of riboflavin are replaced by chlorine atoms. The dichloro compound not only lacks growth



Riboflavin



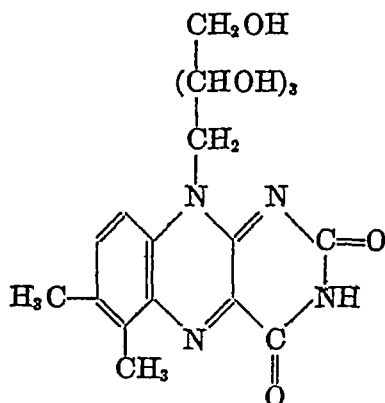
6,7-dichloro-9 ribityl isalloxazine

promoting properties, but effectively interferes with the action of riboflavin, so that the growth of certain bacterial species is blocked. Depending upon the duration of the experiment, the ratio of the concentration of inhibitor to that of metabolite required to permit 50 per cent of normal growth varied from 50:1 to 280:1 with *Staphylococcus aureus*, and from 25:1 to 165:1 for *Streptobacterium plantarum*. It is conceivable that this very active compound will prove useful, possibly as a chemotherapeutic agent, but almost certainly as a tool for the study of the metabolism of riboflavin.

Although the data available concerning this compound are very limited it would appear that its inhibitory effect possibly is *not* limited to those organisms unable to synthesize riboflavin. If this finding be confirmed, the substance may

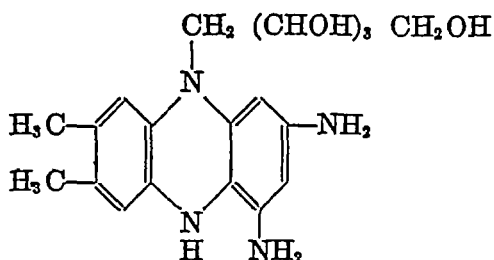
be added to those few compounds or groups of compounds which appear to be effective inhibitors, regardless of the ability of the organism to synthesize the respective metabolite. This group includes, in addition to the dichloro analog of riboflavin, benzimidazole, indoleacrylic acid, and of course, *par excellence*, the antagonists of p-aminobenzoic acid.

Emerson and Tishler (72) have described studies with isoriboflavin, an analog of the vitamin in which a methyl group is moved from the 7-position to the 5-position on the benzene ring. In rats a growth depression is produced by this compound, which is counteracted by an adequate amount of riboflavin. Woolley



5,6-dimethyl-9-ribityl-isoalloxazine  
(Isoriboflavin)

also has described an analog of riboflavin (73), in which the phenazine ring is substituted for the alloxazine ring of the vitamin, a modification which may be considered to involve primarily the replacement, in alloxazine, of the 6-membered pyrimidine ring, containing two N-atoms, with a benzene ring. In

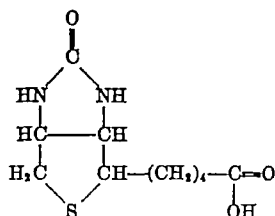


2,4-diamino-7,8-dimethyl-10-ribityl-5,10-dihydrophenazine

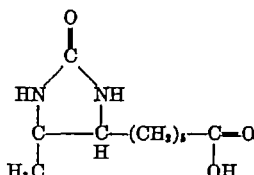
Woolley's compound amino groups also replace the oxygen atoms of alloxazine. This compound causes typical riboflavin deficiency in mice, a condition which is prevented by adding an excess of the vitamin to the diet.

Interesting results with biotin and its desthiobiotin derivative have been reported by duVigneaud and his associates (74, 75) and by Lilly and Leonian (76).

Both groups find that desthiobiotin is for some organisms a growth-factor, while for others it is an antagonist of biotin. It is suggested by the Cornell



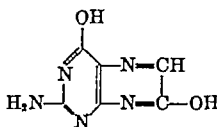
Biotin



Desthiobiotin

group, from their data with *Saccharomyces cerevisiae* in an actively growing state, that, for those organisms for which desthiobiotin is a growth factor, the substance is actually converted into biotin. For *L. casei*, however, and for a few other organisms unable to utilize desthiobiotin, the compound appears to inhibit the action of biotin competitively.

Another example of possible metabolite-antagonism is to be found in the investigations of Wright and Skeggs (77) who observed that the production of "folic acid" (*L. casei* factor) by *Aerobacter aerogenes* is significantly reduced by growing the organism in the presence of synthetic xanthopterin. However, the growth of this microorganism and its production of biotin, are not affected by the pterin.

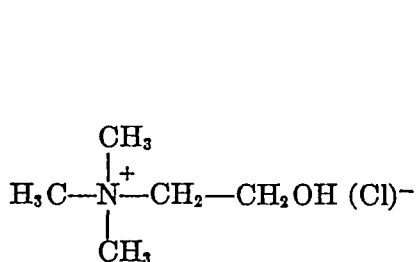


Xanthopterin

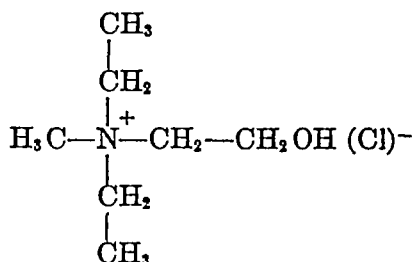
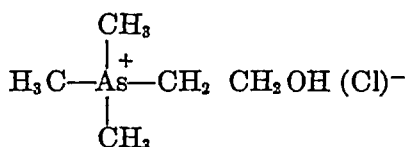
Further evidence of a possible relationship between "folic acid" and xanthopterin is afforded by the observations of Wright, Skeggs and Welch (78) showing that incubation of the hepatic tissue of rats with xanthopterin causes a significant increase in the yield of "folic acid." The evidence presently available indicates that xanthopterin interferes with the inactivation of "folic acid," a reaction presumably catalyzed by an enzyme system (79). Confirmation and explanation of the remarkable observations of Leuchtenberger, et al., on the inhibition of the growth of transplanted tumor tissue in mice by a crystalline "folic acid" (80) or by xanthopterin (81) is not yet available, if this effect of "folic acid" is verified, it is conceivable that the action of xanthopterin may be related to an interference with the destruction of "folic acid" absorbed from the alimentary tract of the animals.

A very large group of choline derivatives has been investigated for activity in preventing the development of fatty livers (82, 83) or of a hemorrhagic disease of the kidneys in mice or rats (84), various choline derivatives have also been examined for activity as avian growth promoting agents and as agents exerting anti perotic activity in chicks (85), and for their ability to substitute for choline

as a growth factor for pneumococci (86) In only a very few cases have observations been made which suggest that any of the phases of the metabolism of choline are interfered with appreciably by compounds of related structure Moyer and duVigneaud (87) observed that diethyl-methyl- $\beta$ -hydroxyethylammonium chloride was somewhat toxic in growth experiments in rats, however, there is evidence that this toxic effect is antagonized by the simultaneous administration of choline (Jukes and Welch, 85) It may be of interest to point out that even



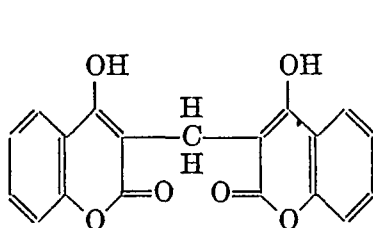
Choline chloride

Diethyl-methyl- $\beta$ -hydroxyethylammonium chloride

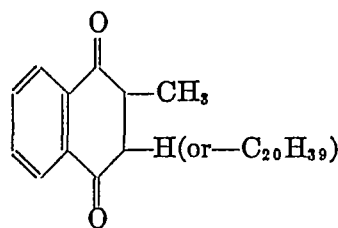
Arsenocholine chloride

the arsenic analog of choline, trimethyl- $\beta$ -hydroxyethylarsonium chloride, does not appear to interfere appreciably with the transmethylating action of choline (88), and actually substitutes for choline in the synthesis of phospholipid (Welch and Landau, 89)

*Antagonism of vitamin K* It has only recently been appreciated that the action of the coumarin derivative, isolated from spoiled sweet clover by Link and his co-workers (90), and now used in medicine to prolong the prothrombin time of blood, is due to its interference with the function of vitamin K In



3,3'-methylene bis(4-hydroxycoumarin)

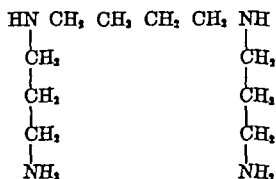


Vitamin K

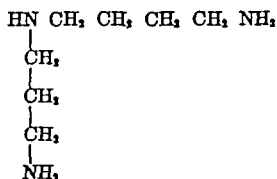
retrospect the structural resemblance between the compounds can be appreciated, and the actual relation is shown by the fact that the effects produced by the coumarin are removed by administration of relatively large amounts of vitamin K

*Antagonism by polyamines* Since the first report by Silverman and Evans

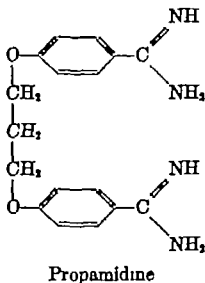
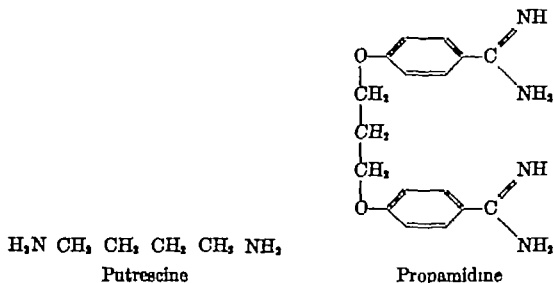
(45), several workers have studied the effect of various polyamines on the bacteriostatic influence of atabrine or propamidine (Snell, 91, Miller, Peters and Bosshardt, 92) There is no doubt that the action of atabrine or of propamidine on *E. coli*, and on some other organisms, is markedly reduced in the presence of tetraethylenepentamine, triethylenetetramine, spermine or spermidine, while the chemically related compound, putrescine, is inactive The effects of these



Spermine



Spermidine



Propamidine

polyamines are exhibited best within a certain range of concentration and in certain media. Whether they can be accounted for on the basis of competitive antagonism is not yet altogether clear. It is not inconceivable, however, that the compounds are related in structure to some substance thus far unidentified, the action of which is interfered with by atabrine and propamidine.

**DISCUSSION** The number of examples of metabolite-antagonism is now so great, the nature and function of the various metabolites so diverse, and the ways of modifying their structure to form antagonists so numerous, that the principle of interference with biological processes through the use of analogs of essential metabolites must now be considered as established. In some cases the interference can be explained most simply on the basis of a direct competition between the metabolite and its analog for some cellular component for which they both have great affinity, in other cases the data indicate that, in addition to competition, other factors operate. In the antagonism of p-aminobenzoic acid, and apparently in a few less well-defined cases, antagonism by related structures extends to metabolites normally synthesized by the organism whose function is affected. However, the majority of the interferences so far investigated



involve those forms of life unable to synthesize the essential function of which is disturbed

An explanation of the too common failure of analogs to biologically endogenously formed metabolites is badly needed. Partly because the newer concepts have not led as yet to the development of analogs of practical importance. There is still reason to believe, however, that the approach to the discovery of new drugs is rational, and progress is being formulated that will lead almost certainly to great results. It would be curious indeed if man, as a result of the groping work in the discovery of the sulfonamides, should have found first the only group of practical utility in the antagonism of an essential metabolite, that is not the case, let us examine the prospects for the future.

There are many avenues of approach which have not yet been explored. Practically every important growth factor or essential metabolite has only a few analogous compounds have been prepared, among which the chemist can conceive and which the chemist may find a way to synthesize. Many more derivatives must be made before rules relating chemical structure to antagonistic action can be formulated with any degree of precision. It is not unlikely that rules in most cases will largely be restricted to one essential metabolite or to a group of metabolites having common features. However, a few concepts of general significance concerning the relation of structure to the development of antagonistic properties are being developed.

The conversion of a carboxyl group to a sulfonic acid group or of an unsubstituted sulfonic acid amide has been particularly profitable. One example of this modification may be seen, of course, in the analogs of ascorbic acid, nicotinic acid and pantothenic acid. The carboxyl group has been changed to the  $-\text{CO}-\text{R}$  group, as in *p*-aminoacetophenone and pyridine. Isosteric modifications in which a ring  $-\text{CH}=\text{C}-$  is changed to  $-\text{S}-$ , or *vice versa*, appear to be promising from the results with pyrimidine and thienylalanine, although in the paba field this modification has not led to particularly active structures, except in the nitro derivatives (25).

Many other possibilities for chemical modification remain to be explored. These beginnings have been made. For example, the shift of functional groups, or their replacement with halogen, as in a modification of the lengthening of a chain, as with ascorbic acid, the replacement of oxygen with those of sulfur, as might be done in hydroxyl groups, carbonyl and amide linkages, in general, and a change in the reverse direction in some cases, the replacement of N with C, P, As, Sb, Se, Te, S or O, the replacement of C with N or O, the introduction of alkyl or other groups into the side chain, and other modifications, apparent to the chemist, but too numerous to list. The important point to appreciate is that blocking-agents can be made in a variety of ways but as yet no predictions can safely be made as to the effect of any given modification of chemical structure on the activity of a biologic-analog.

The information which is steadily being acquired is of the greatest significance to chemotherapy in particular and to biological sciences in general. Of specific significance should be structural modifications of those essential metabolites active in the smallest amounts. With such compounds we may hope that certain microorganisms will prove to be relatively more dependent upon the essential metabolite than is the infected host. It may be suggested, from this line of reasoning, that analogs of biotin or of "folic acid" may be of promise and there is reason to believe that extensive work in the field of biotin chemistry, with such a goal in view, is now in progress.

Another field, separate from, but related to metabolite antagonism, is developing in pharmacology. This concerns the further structural modification of compounds with known involvement in, or with influence on various physiological processes. It is readily apparent that the effective blocking of the action of many hormones and drugs might have great practical utility. It is not improbable that certain drugs exert all or a portion of their desirable or undesirable effects on bodily mechanisms through competitive antagonism. It will suffice to comment upon a report by Swan and White (93) concerning certain compounds related to acetylcholine that act in the opposite manner, and function, therefore, as mydriatics and cycloplegics. Also Unna (94) has reported on the use of N allyl morphine in allaying the respiratory depression and other manifestation of toxicity of morphine. Although it is thought that the effect of atropine cannot be explained simply on the basis of competition with acetylcholine released at the endings of cholinergic nerves, a competitive type of antagonism appears to exist between pilocarpine and atropine, a phenomenon clearly demonstrated by Cushny a generation ago.

All the findings described emphasize that within the body of an animal the innumerable problems offered by the processes of absorption, distribution, excretion, metabolic alteration and toxicity, make our search for effective anti-metabolites, particularly chemotherapeutic agents, a much more complicated task than the discovery of compounds with inhibitory properties that are counteracted by the corresponding physiological substance. Despite these difficulties it is reasonable to hope that the concept of competitive inhibition will be responsible for many useful developments in various fields of significance to biology and medicine. Application of these new principles permits the prediction that somewhat more in the future than in the past, and somewhat more by design than by chance, may we anticipate the discovery of new tools for the investigation of cellular metabolism and the treatment of disease.<sup>3</sup>

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<sup>3</sup> The writer acknowledges with pleasure the contribution of many friends with whom he has discussed the subject of this paper and who have commented on earlier versions of the manuscript. Among others these include Drs L E Arnow, E A Evans Jr, E E Snell, J M Sprague, D W Woolley and L D Wright. However, only the writer should be held responsible for the manner of presentation and for any errors of omission or of commission. The reader is also referred to a recent excellent shorter article by Dr Woolley on the subject discussed herein. This appeared in *Science* 100 579, 1944.

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